

**You might find this additional information useful...**

This article cites 392 articles, 217 of which you can access free at:  
<http://physrev.physiology.org/cgi/content/full/82/1/97#BIBL>

This article has been cited by 45 other HighWire hosted articles, the first 5 are:

**TLR/MyD88 and Liver X Receptor {alpha} Signaling Pathways Reciprocally Control Chlamydia pneumoniae-Induced Acceleration of Atherosclerosis**

Y. Naiki, R. Sorrentino, M. H. Wong, K. S. Michelsen, K. Shimada, S. Chen, A. Yilmaz, A. Slepentin, N. W. J. Schroder, T. R. Crother, Y. Bulut, T. M. Doherty, M. Bradley, Z. Shaposhnik, E. M. Peterson, P. Tontonoz, P. K. Shah and M. Arditi  
*J. Immunol.*, November 15, 2008; 181 (10): 7176-7185.  
[Abstract] [Full Text] [PDF]

**Maturation of dendritic cells depends on proteolytic cleavage by cathepsin X**

N. Obermajer, U. Svajger, M. Bogyo, M. Jeras and J. Kos  
*J. Leukoc. Biol.*, November 1, 2008; 84 (5): 1306-1315.  
[Abstract] [Full Text] [PDF]

**Cigarette Smoke Exposure Impairs Dendritic Cell Maturation and T Cell Proliferation in Thoracic Lymph Nodes of Mice**

C. S. Robbins, F. Franco, M. Mouded, M. Cernadas and S. D. Shapiro  
*J. Immunol.*, May 15, 2008; 180 (10): 6623-6628.  
[Abstract] [Full Text] [PDF]

**Phase I Study and Preliminary Pharmacology of the Novel Innate Immune Modulator rBBX-01 in Gynecologic Cancers**

J. S. Rader, C. F. Aylsworth, D. A. Juckett, D. G. Mutch, M. A. Powell, L. Lippmann and N. V. Dimitrov  
*Clin. Cancer Res.*, May 15, 2008; 14 (10): 3089-3097.  
[Abstract] [Full Text] [PDF]

**Physiology of the Prion Protein**

R. Linden, V. R. Martins, M. A. M. Prado, M. Cammarota, I. Izquierdo and R. R. Brentani  
*Physiol Rev*, April 1, 2008; 88 (2): 673-728.  
[Abstract] [Full Text] [PDF]

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Oncology .. Dendritic Cells  
Oncology .. Adjuvants  
Oncology .. Vaccine Development  
Oncology .. CD4  
Oncology .. Lymph  
Oncology .. Spleen

Updated information and services including high-resolution figures, can be found at:  
<http://physrev.physiology.org/cgi/content/full/82/1/97>

Additional material and information about *Physiological Reviews* can be found at:  
<http://www.the-aps.org/publications/prv>

This information is current as of December 18, 2008 .

# Dendritic Cells: Immune Regulators in Health and Disease

MARY F. LIPSCOMB AND BARBARA J. MASTEN

*Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico*

I. Introduction	97
II. Dendritic Cell Origins	98
A. Murine DCs	98
B. Human DCs	100
III. Differentiation and Trafficking Pathways	101
IV. Dendritic Cell-T Cell Interactions	104
A. Antigen uptake	104
B. Antigen processing	104
C. Costimulation	106
D. Events at the DC-T cell interface	108
V. Role of Dendritic Cells in Linking Innate and Acquired Immunity	108
A. Microenvironment influences DC phenotype and function: recognizing danger	108
B. DC role in T cell memory, effector function, and tolerance	110
C. DC role in B cell function	112
VI. Dendritic Cells and Infectious Disease	112
A. Microbial subversion of DC function	112
B. Exploiting DCs for vaccine protocols	114
VII. Role of Dendritic Cells in Immune-Mediated Disease	116
A. Asthma: a Th2-mediated inflammatory lung disease	116
B. EAE: a Th1-mediated disease	118
VIII. Role of Dendritic Cells in Transplantation With Therapeutic Implications	118
IX. Role of Dendritic Cells in Cancer With Therapeutic Implications	120
X. Summary and Future Directions	120

**Lipscomb, Mary F., and Barbara J. Masten.** Dendritic Cells: Immune Regulators in Health and Disease. *Physiol Rev* 82: 97–130, 2002; 10.1152/physrev.00023.2001.—Dendritic cells (DCs) are bone marrow-derived cells of both lymphoid and myeloid stem cell origin that populate all lymphoid organs including the thymus, spleen, and lymph nodes, as well as nearly all nonlymphoid tissues and organs. Although DCs are a moderately diverse set of cells, they all have potent antigen-presenting capacity for stimulating naive, memory, and effector T cells. DCs are members of the innate immune system in that they can respond to dangers in the host environment by immediately generating protective cytokines. Most important, immature DCs respond to danger signals in the microenvironment by maturing, i.e., differentiating, and acquiring the capacity to direct the development of primary immune responses appropriate to the type of danger perceived. The powerful adjuvant activity that DCs possess in stimulating specific CD4 and CD8 T cell responses has made them targets in vaccine development strategies for the prevention and treatment of infections, allograft reactions, allergic and autoimmune diseases, and cancer. This review addresses the origins and migration of DCs to their sites of activity, their basic biology as antigen-presenting cells, their roles in important human diseases and, finally, selected strategies being pursued to harness their potent antigen-stimulating activity.

## I. INTRODUCTION

Dendritic cells (DCs) were first described in the mid 1970s by Ralph Steinman, who observed in the spleen a subpopulation of cells with a striking dendritic shape. These cells were nonphagocytic, loosely adherent, and of

low buoyant density (325–327). It was soon appreciated that these bone marrow-derived cells existed in all lymphoid and most nonlymphoid tissues. DCs were described as cells that constitutively expressed both major histocompatibility complex (MHC) class I and class II antigens, spontaneously clustered T cells via antigen-independent

mechanisms (later understood to represent the interplay of surface molecules on DCs that were mutually complementary to surface molecules on T cells), and, most importantly, stimulated naive CD4 and CD8 T cells to respond to nominal and alloantigens more effectively than any other previously described antigen presenting cell (APC).

In recent years, DCs have been increasingly studied for their role as critical adjuvants in vaccines for prevention of microbial infection and allograft rejection and treatment of cancer and autoimmune diseases. Several reviews on DCs and their role in immune regulation have appeared recently, because of the increased realization of their importance in immunoregulation and possibilities for exploiting them for biomedical purposes (Refs. 21, 22, 134, 188 are representative). This review overviews DC biology, highlighting more recent literature. DC origins and differentiation pathways are discussed, including factors that regulate their migration to sites where they play their surveillance role. How DCs link innate and adaptive immunity will be reviewed, with a separate section on how certain pathogens, to survive in the infected host, subvert the immunostimulating activity of DCs. The role DCs play in autoimmune and allergic diseases, transplantation, and cancer is described. In relevant sections, representative studies that have manipulated DCs for therapeutic purposes are summarized.

## II. DENDRITIC CELL ORIGINS

DCs are a heterogeneous group of cells that display differences in anatomic localization, cell surface phenotype, and function. However, DCs have several features in common (22, 134). First, originating from CD34 bone marrow stem cells, precursor DCs are seeded via the bloodstream to the tissues where they give rise to immature DCs that include Langerhans cells (LCs) and interstitial DCs (also called dermal DCs). Second, immature DCs have the ability to take up antigen, via both receptor- and non-receptor-mediated mechanisms, and readily degrade antigens in endocytic vesicles to produce antigenic peptides capable of binding to MHC class II. Third, in response to danger signals, i.e., tissue damage, pathogen-derived products, or inflammatory cytokines, DCs mature and migrate to lymphoid organs where they interact with antigen-specific CD4 T cells to initiate immune responses (83, 169, 183, 205, 257, 362, 366). Fourth, distinct chemokine receptors occur on immature DCs, compared with mature DCs, which regulate their traffic into tissue sites in response to inflammatory chemokines (71, 143, 283, 396). Fifth, as DCs mature, they express a high density of MHC class II molecules complexed with antigen for recognition by the T cell receptor (TCR) expressed on CD4 T cells and costimulatory molecules to stimulate CD4 T cell prolifer-

ation. Finally, other factors in the microenvironment at the time of DC maturation have been shown to dictate whether DCs will produce IL-12 and initiate Th1 responses or have their IL-12-producing capacity suppressed and initiate Th2 responses (366).

DCs generally have a low buoyant density and are initially adherent to plastic but then readily detach (227, 329). Early methods of DC isolation used enzyme-digested tissue and exploited these attributes to obtain enriched populations of DCs from murine tissue. Selection of low-buoyant-density cells enriches for mononuclear cells, and the adherence step helps eliminate T cells and B cells from the preparation. Further enrichment of DCs utilizes various combinations of additional negative and positive selection steps. One negative selection step is based on phagocytosis of silica particles, latex beads, or carbonyl iron particles to remove avidly phagocytic macrophages from preparations. Other negative selection steps eliminate contaminating B cells, T cells, and NK cells from the preparation by a combination of immunophenotyping and cell sorting. Positive selection steps use various combinations of monoclonal antibodies to isolate cells expressing important DC cell surface markers, typically MHC class II and CD11c. With the use of multiple enrichment steps, pure populations of tissue-derived DCs have been obtained.

DCs can also be propagated from bone marrow and blood using various combinations of growth factors, such as granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-4, stem cell factor (SCF), transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-3, and Flt3 ligand (Flt3L) (47, 151, 221, 264, 279, 283, 332, 392). GM-CSF in combination with IL-4 or TNF- $\alpha$  and other cytokines provides important growth factors for interstitial DCs and LCs. In addition, LCs also require TGF- $\beta$  for their differentiation (35, 332). IL-3 is a cytokine required by plasmacytoid DCs, a noninterstitial, non-LC DC subtype, that express CD123 (IL-3 R $\alpha$ ) and are found in lymphoid tissue (127). Flt3L has been used to stimulate the proliferation of stem cells and progenitor cells *in vitro* and expand and mobilize all DCs and their progenitors *in vivo* (221, 222, 262, 264).

### A. Murine DCs

Murine DCs have been classified into two main lineages: myeloid DCs as originally described by Steinman and Cohn (327) and lymphoid DCs described by Suss and Shortman (339). However, researchers now recognize that enrichment steps led to selective loss of DC subpopulations and that murine DCs are not readily separated into these two distinct lineages. A current method to obtain DCs that avoids depletion of DC subpopulations involves mild collagenase digestion; breaking DC-T cell complexes

with EDTA; selecting low-density cells; depleting T cells (CD3+ or Thy1+), B cells (B220+), granulocytes (Gr1+), and erythroid lineage cells (TER-119+) by immunomagnetic bead depletion; and finally positive sorting for cells expressing CD11c and MHC class II (226, 368). DCs can be further segregated into subtypes based on expression of CD4 and the CD8 $\alpha$  chain homodimer (CD8 $\alpha\alpha$ ), markers originally thought to be confined mainly to T cells (226, 368). Two important problems in subtyping DCs based on expression of CD4 and CD8 $\alpha\alpha$  are autofluorescence and adsorption of CD4, CD8 $\alpha\beta$ , and Thy-1 surface antigens from other cells (368). Other difficulties that further confuse the subtyping of DCs are the different stages of maturation that DCs exhibit *in situ*, i.e., bone marrow progenitors, precursor DCs in blood and lymphatics, immature DCs in tissue, and mature DCs within secondary lymphoid organs. Thus, in evaluating the literature on DC subtypes, the procedure used for isolation, the controls used to minimize immunofluorescent staining artifacts, the authenticity of surface CD4 and CD8 $\alpha\alpha$  markers, and the developmental state of the DC must all be considered.

Over the past few years, numerous reports detailing different isolation procedures and dealing with the phenotype, localization, and function of murine CD8 $\alpha$ + and CD8 $\alpha$ - DCs have been published, contributing to our understanding of DC biology (125, 170, 178, 179, 195, 219, 251, 263, 264, 269, 280, 305, 339, 368, 378, 381, 382). At least five major populations of DCs have been described in the central and peripheral lymphoid organs of mice (see Table 1). In murine spleen, three DCs subtypes are delineated, namely, CD4-8 $\alpha$ +DEC205+CD11b-, CD4+8 $\alpha$ -DEC205-CD11b+, and CD4-8 $\alpha$ -DEC205-CD11b+ (170, 305, 368, 381). In lymph nodes, these three subtypes are present together with a fourth population, CD4-8 $\alpha$ (lo)DEC205+ with various levels of CD11b- (11, 305). The mouse thymus appears to

contain two DC types, one that overlaps with a lymph node subtype and one that may be unique, CD4-CD8 $\alpha$ -/loDEC205+CD11b- and CD4-CD8 $\alpha$ -DEC205+CD11b-, respectively. Based on phenotype and maturation kinetics, CD4-bearing DC depletion studies, bromodeoxyuridine (BrdU) labeling kinetics, and bone marrow reconstitution studies, the three spleen DC subtypes appear to be products of three independent developmental streams, not different states of maturation. All three subtypes were classed as mature, because they expressed CD80, CD86, and CD40 and efficiently activated allogeneic T cells (368). However, further maturation was induced in all these subtypes by bacterial stimuli (170). Contradictory to conventional views about DC maturation was that these three DC subtypes phagocytosed particulate material *in vivo* and upon maturation retained phagocytic capacity. Upon maturation, no DC subtype converted to the other, and continuous elimination of CD4-bearing DCs by antibody depletion had no effect on numbers of the other two DC subtypes. BrdU labeling experiments indicated all three DCs subtypes had a rapid turnover in the spleen, with the CD4-CD8 $\alpha$ + DCs showing the fastest turnover and with none being the precursor of the other. Immunofluorescent staining of spleen sections showed that the two CD8 $\alpha$ - populations, i.e., CD4+8 $\alpha$ - and CD4-8 $\alpha$ - DCs, were in the marginal zones of the spleen, with only CD4-8 $\alpha$ + DCs concentrated in T cell areas. However, in response to microbial stimuli such as lipopolysaccharide (LPS), CD8 $\alpha$ - DCs rapidly migrated to T cell areas (82). Not delineated in this microbial stimuli study were the migratory responses of CD4+ and CD4- subsets of the CD8 $\alpha$ - DC population. Functionally CD8 $\alpha$ + and CD8 $\alpha$ - appeared distinct with CD8 $\alpha$ + DCs producing much higher levels of IL-12 than the CD8 $\alpha$ - DCs *in vitro* (179, 218, 265). Whether this distinction holds true *in vivo* when

TABLE 1. Murine DC subsets in the spleen, lymph nodes, and thymus

	Dendritic Cell Subsets				
	CD4+8 $\alpha$ -	CD4-8 $\alpha$ +	CD4-8 $\alpha$ +	CD4-8 $\alpha$ -/low	CD4-8 $\alpha$ +
Phenotype					
CD11c	+	+	+	+	+
CD11b	+	-	+	-	-
CD80	+	+	+	+	+
CD86	+	+	+	+	+
CD40	Low	Low	Low	+	+
MHC class II	+	+	+	+	+
DEC205	-/Low	+	-/Low	+	+
F4/80	+	-	+	-	-
Function					
Phagocytic ability	Yes	Yes	Yes		
Primes CD4/CD8 T cells	Yes	Yes	Yes		
Tissue	Spleen	Spleen	Spleen	Thymus	Thymus
	Lymph nodes	Lymph nodes	Lymph nodes	Lymph nodes	
Localization	Marginal zones of spleen	T cell areas of lymphoid tissue	Marginal zones of spleen	T cell areas of lymph nodes	Thymic cortex

DC, dendritic cell; MHC, major histocompatibility complex. Synthesis from Refs. 74, 84, 170, 179, 180, 218, 221, 226, 263, 291, 305, 368, 381.

the two CD8 $\alpha$ <sup>+</sup> and the one CD8 $\alpha$ <sup>−</sup> subsets are evaluated as three subsets, i.e., CD4<sup>+</sup>8 $\alpha$ <sup>−</sup>, CD4<sup>−</sup>8 $\alpha$ <sup>+</sup>, and CD4<sup>−</sup>8 $\alpha$ <sup>−</sup>, must be determined.

The question remains whether the subsets of DCs have a common progenitor. Because CD8 $\alpha$ <sup>+</sup> DCs lack the myeloid marker CD11b, they were originally thought to arise from a lymphoid-committed progenitor and were generated at low frequencies from thymic T cell progenitors (13). In contrast, CD8 $\alpha$ <sup>−</sup> DCs, which may be either CD4<sup>+</sup> and CD4<sup>−</sup>, generally express CD11b, were considered myeloid related, and could be derived from myeloid progenitors (150). Additional arguments were made that the CD8 $\alpha$  marker on DCs reflected an origin from a precursor different from the CD8 $\alpha$ <sup>−</sup> populations, because CD8 $\alpha$ <sup>+</sup> DCs have distinct cytokine requirements for their *in vitro* generation and utilize different transcription factors (293, 380). For example, thymic CD8 $\alpha$ <sup>−</sup> DCs precursors require GM-CSF for differentiation in culture. In contrast, CD8 $\alpha$ <sup>+</sup> DCs precursors require IL-3, but not GM-CSF, to differentiate (293). The absence of CD8 $\alpha$ <sup>−</sup> and the presence of CD8 $\alpha$ <sup>+</sup> DCs in RelB and PU.1 knock-out mice suggest RelB and PU.1 play a role in the development of CD8 $\alpha$ <sup>−</sup> DCs (129, 380). A recent study assessed the ontogeny of CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>−</sup> DCs. Traver et al. (357) showed by transfer of marked, lineage-restricted progenitors that both CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>−</sup> DCs arise from common myeloid and lymphoid progenitors in both murine thymus and spleen. In addition, RelB and PU.1 were expressed in both CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>−</sup> DCs. Clearly, DCs can be derived from either myeloid or lymphoid precursors. However, the study by Traver et al. (357) indicates that CD8 $\alpha$  on DCs does not indicate a lymphoid origin but rather may reflect the maturation or differentiation status and once defined may predict the function of the DC.

## B. Human DCs

In humans, DCs are also found as precursor populations in bone marrow and blood and as more mature forms in lymphoid and nonlymphoid tissues. Three distinct subtypes of human DCs have been delineated based on studies of skin DCs (57), DCs generated *in vitro* from CD34<sup>+</sup> hematopoietic progenitors (51), and blood DC precursors (see Fig. 1) (279). Human skin contains two of the three DC subtypes in immature form: LCs and interstitial DCs. Both subtypes emerge in cultures from CD34<sup>+</sup> bone marrow and CD11c<sup>+</sup> blood precursors in the presence of GM-CSF and either IL-4 or TNF- $\alpha$  (48, 279, 283). The CD11c<sup>+</sup> DC precursor expresses myeloid markers, including CD13 and CD33. Upon activation by CD40L, immature myeloid DCs undergo maturation and produce IL-12 (56). A distinction from interstitial DCs is that LCs also require TGF- $\beta$  (154) and arise from either a

CD11c<sup>+</sup>CD14<sup>+</sup> monocyte or a CD11c<sup>+</sup>CD14<sup>−</sup> precursor, whereas interstitial DCs arise from a CD11c<sup>+</sup>CD14<sup>+</sup> precursor that can also differentiate into macrophages in the presence of only M-CSF (48, 279, 283, 393). The demonstration that LCs can arise from CD11c<sup>+</sup>CD14<sup>+</sup> monocyte is controversial and may ultimately reflect the plasticity of DCs. LCs and interstitial DCs subtypes share several markers, but LCs uniquely express CD1a, Birbeck granules, langerin, and the adhesion molecule E-cadherin. In contrast, interstitial DCs uniquely express the coagulation factor XIIIa. LCs and interstitial DCs also share the capacity to activate both CD4 and CD8 naive T cells and secrete IL-12. One striking difference between LCs and interstitial DCs is the ability of interstitial DCs, but not LCs, to induce the differentiation of naive B cells into immunoglobulin-secreting plasma cells (49, 92).

Plasmacytoid DCs are a third type of DC and are so named because at the ultrastructural level they resemble Ig-secreting plasma cells. These DCs are found in the T cell zones of lymphoid organs and in the thymus and blood and were previously described as plasmacytoid T cells or plasmacytoid monocytes (98, 127, 249, 272, 314, 333). Plasmacytoid DCs are characterized by a unique phenotype, CD11c<sup>−</sup>CD4<sup>+</sup>CD123<sup>+</sup>CD45RA<sup>+</sup>HLA-DR<sup>+</sup>, and possess the unique ability to secrete large amounts of interferon (IFN)- $\alpha$ /B upon viral stimulation (54, 127, 174, 307). In this context, precursor plasmacytoid DCs in blood correspond to natural IFN- $\alpha$ -producing cells, suggesting an important role during viral infections (33, 292). Precursor plasmacytoid DCs in blood express CD62L and the chemokine receptor CXCR3, which mediate homing and migration of these cells into the lymph node via high endothelial venules (HEVs) in response to inflammatory chemokines (54). Unlike LCs and interstitial DCs, plasmacytoid DCs require IL-3 for their differentiation and are derived from a CD11c<sup>−</sup> blood precursor that has low expression of GM-CSF receptor, lacks the myeloid markers CD14, CD13, and CD33, lacks mannose receptors, and expresses high amounts of CD123 (127, 174). Plasmacytoid DCs share a common function with LCs and interstitial DCs in having the capacity to activate CD4 and CD8 naive T cells and secrete IL-12 upon CD40L activation (53, 174). Several lines of evidence suggest that plasmacytoid DCs originate from a lymphoid precursor. First, precursor plasmacytoid DCs lack expression of myeloid antigens (127). Second, precursor plasmacytoid DCs express pre-TCR- $\alpha$  transcripts (42, 272). Third, ectopic expression of inhibitor of DNA binding (Id)2 and Id3 inhibits the development of CD34<sup>+</sup> progenitor cells into CD123<sup>+</sup> precursor plasmacytoid DCs, T and B cells, but not myeloid DCs (320). Finally, precursor plasmacytoid DCs express the immunoglobulin-like transcript receptor (ILT)3, in contrast to myeloid DCs that express both ILT3 and ILT1.

Murine DCs have been widely employed by researchers investigating the roles of DCs in the generation and

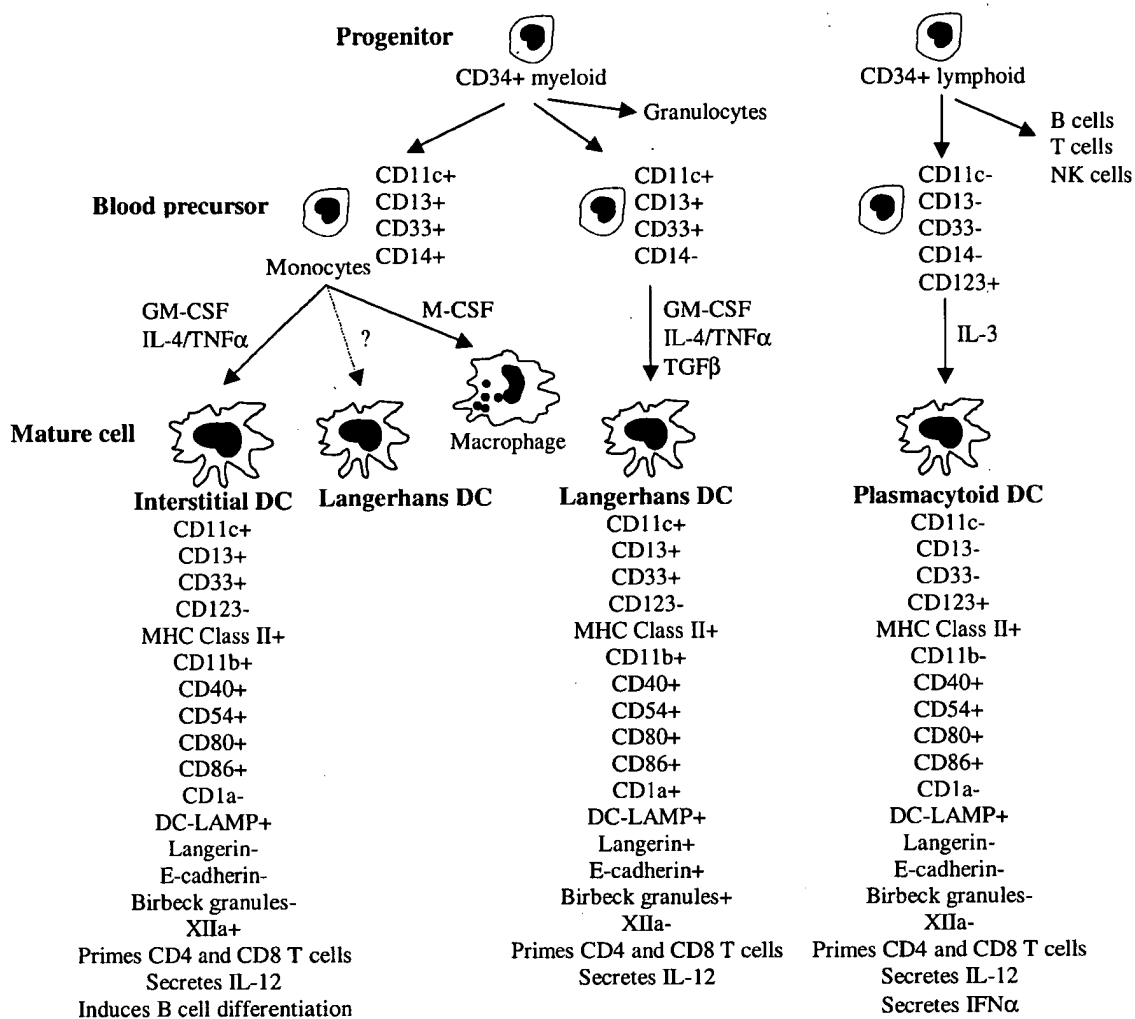


FIG. 1. Schema for derivation of human dendritic cell (DC) subsets from CD34+ myeloid and lymphoid progenitors. In humans, DCs are found as precursor populations in bone marrow and blood and as more mature forms in lymphoid and nonlymphoid tissues (*top*). Both myeloid and lymphoid lineage DCs can be propagated from bone marrow progenitors and blood precursors using various combinations of growth factors, such as granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-4, transforming growth factor- $\beta$  (TGF- $\beta$ ), and IL-3. Interstitial DCs and Langerhans DCs subsets (*middle*) are found at sites that interface with the external environment. Plasmacytoid DCs are found in the T cell zones of lymphoid organs and in the thymus and blood. Shown below each DC subset are phenotype and functional characteristics defining that DC subset. See text for additional details. MHC, major histocompatibility complex; IFN, interferon.

regulation of specific immunity. Although it is clear that differences exist between murine and human DC, it is evident that murine DCs are relevant to human DCs and provide an appropriate model for human cells in most cases. Like human DCs, murine DCs 1) originate from CD34+ bone marrow stem cells, 2) are found in blood and tissues, 3) are able to take up and degrade antigen to antigenic peptides, 4) express MHC class II molecules complexed with antigenic peptide, 5) express costimulatory molecules, 6) mature and migrate in response to danger signals, and 7) are responsive to the microenvironment with a controlled release of chemokines and cytokines. Data from murine DC studies should be inter-

preted with caution in cases where clear discrepancies exist between murine and human subtype counterparts.

### III. DIFFERENTIATION AND TRAFFICKING PATHWAYS

DCs are migratory cells that traffic from one site to the next, performing specific functions at each site (273, 284, 287, 396). Bone marrow-derived DCs circulate as precursors in blood before entering tissue where they become resident immature DCs that monitor their environment (Fig. 2). Interstitial DCs and LCs are found at

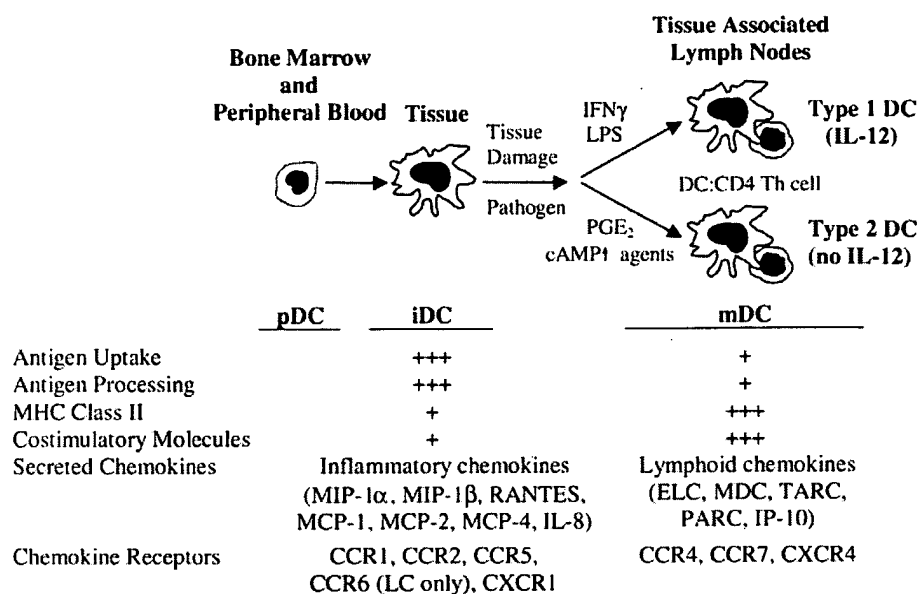


FIG. 2. The migratory and maturation pathways of DCs. DCs are migratory cells that traffic from one site to the next, acquiring specific abilities at each site and performing specific functions in a stepwise fashion. See text for additional details. pDC, precursor DC; iDC, immature DC; mDC, mature DC.

sites that interface with the external environment, i.e., mucosal surfaces and in the skin. In peripheral tissues, immature DCs have the ability to migrate toward inflammatory foci where they take up and process available antigens and then emigrate through the lymphatics to draining lymph nodes. There they home to T cell-rich areas and interact with T cells to initiate an immune response. It has been proposed that the origin of lymphatic-borne DCs may be blood monocytes (242, 268). Using an in vitro model of transendothelial trafficking, Randolph et al. (268) observed that monocytes matured into DCs as they migrated across endothelium from the abluminal to the luminal surface in a manner mimicking entry into the afferent lymphatics. Monocytes that remained in the subendothelial matrix became macrophages and lost migratory capability. In contrast, plasmacytoid DCs are thought to migrate directly from the blood to lymphoid tissue (22, 54).

The extravasation of DCs from the blood to peripheral tissue and the movement from peripheral tissue into lymphoid tissue requires chemoattractants called chemokines. Chemokines are peptide activators of G protein-coupled receptors expressed on leukocytes that regulate recruitment of inflammatory cells (182, 235, 278). Chemokines are differentially produced at peripheral tissue sites by endothelial cells, epithelial cells, and leukocytes in response to diverse inflammatory stimuli. Chemokines are also constitutively produced by endothelial cells or stromal cells and leukocytes within secondary lymphoid organs to regulate encounters between DC, T cells, and B cells (20, 70). The anatomic location of inflammatory chemokines within peripheral tissue and constitutive chemokines within lymphoid tissue regulates the migration of DCs initially to sites of antigen and ultimately to lymphoid tissue to initiate an immune response.

The ability of DCs to respond to inflammatory and lymphoid chemokine gradients is presumably linked to their maturation state, because as DCs mature they lose responsiveness to inflammatory chemokines and gain responsiveness to lymphoid chemokines. Both human monocytes and monocyte-derived immature DCs and murine CD34<sup>+</sup>-derived immature DCs express both CC and CXC chemokine receptors (CCR and CXCR), such as CCR1, CCR2, CCR5, and CXCR1, and respond to inflammatory chemokines such as macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and regulated on activation normal T cell expressed and secreted (RANTES) chemokine (87, 289, 315, 318, 319, 364). In addition, monocytes and immature DCs express chemoattractant receptors for cleavage products of bacterial proteins such as formyl-methionyl-leucyl-phenylalanine (fMLP), products of host complement activation, such as C5a, and lipid metabolites, such as platelet activating factor (PAF) (9, 239, 317, 319, 385). As immature DCs migrate toward increasing concentrations of inflammatory chemokines, they are also exposed to increasing concentrations of proinflammatory cytokines, such as TNF- $\alpha$  and IL-1 and the pathogen products initiating the inflammatory response. In response to these danger signals, DCs mature, and in doing so switch the usage and expression of chemokine receptors from inflammatory to lymphoid homing receptors. The loss of inflammatory chemokine receptors is at least partly regulated by ligand-induced downregulation by autocrine secretion of MIP-1 $\alpha$ , MIP-1 $\beta$ , and RANTES by maturing DCs (288). Maturing DCs downregulate the expression of CCR1, CCR5, and CXCR1 and upregulate the expression of CXCR4, CCR4, and, in particular, CCR7, a chemokine receptor that responds to secondary lymphoid tissue che-

mokine (SLC) and Epstein-Barr virus-induced ligand chemokine (ELC). SLC is produced by lymphatic endothelial cells, and both SLC and ELC are produced by stromal cells and DCs in the T cell areas of lymphoid organs (85, 87, 108, 130, 198, 247, 315, 364, 388). The anatomic distribution of SLC and ELC secretion coordinately attracts DCs first from peripheral tissue to afferent lymphatics and then to T cell areas in lymphoid tissue. CCR7 is also selectively expressed on naive T and B lymphocytes, allowing these cell types to also home to lymphoid tissue (45). CCR7 is a unique chemokine receptor, because it is resistant to ligand-induced downregulation (288). Sustained expression of CCR7 may allow DCs to perform their stepwise migration from tissue to afferent lymphatics to the lymphoid organ. The essential role of CCR7 in DC homing to lymphoid organs is supported by the observation that in CCR7-deficient mice, maturing DCs are not able to migrate to lymph nodes (107).

Upon maturation, bacterial and complement receptors on DCs are also differentially regulated. Maturing DCs downregulate their responsiveness to and receptor expression for fMLP, while maintaining their responsiveness to and receptor expression of C5a (385). Yang et al. (385) proposed that the interaction of C5a with C5aR on mature DCs may participate in guiding mature DCs to B cell follicles in lymphoid tissue where naive B cells, a source of C5a, acquire antigens delivered by mature DCs.

LCs are unique in that they express CCR6 in addition to other chemokine receptors expressed by immature DCs (85, 86, 316). CCR6 is a chemokine receptor that responds to MIP-3 $\alpha$  produced constitutively by epithelial cells in human liver and lung, induced in the crypts of inflamed tonsils and appendix in humans, and produced in noninflamed follicle-associated epithelium of murine Peyer's patches (63, 86, 155). MIP-3 $\alpha$  and CCR6 represent a chemokine/receptor pair that has dual function in recruiting DCs. Not only does this pair recruit immature DCs to mucosal and nonmucosal sites of inflammation, but also recruits immature DCs to become sentinels in noninflamed tissues. As LCs mature, CCR6 is downregulated with a concomitant upregulation of CCR7 and homing to T cell areas of lymphoid tissue.

During the course of an inflammatory reaction, DCs produce inflammatory and lymphoid chemokines in a specific spatial and time-ordered manner (108, 284, 287). Immature DCs constitutively produce MCP-4 that binds to both CCR2 expressed on immature DCs and to CCR3, a potential marker for a subset of Th2 cells (286). In response to maturation stimuli, the production of MCP-4 by DCs is rapidly downregulated, and the inflammatory chemokines, MIP-1 $\alpha$ , MIP-1 $\beta$ , and IL-8 are transiently induced for a few hours. Other inflammatory chemokines such as RANTES, MCP-1, and MCP-2 are also induced, but for a longer period of time. The inflammatory chemokines produced by maturing DCs function in both autocrine and

paracrine modes to regulate DC trafficking. In an autocrine mode, they initially stimulate, then downregulate, cognate receptors allowing the DC to respond to other chemoattractants. In a paracrine mode, DCs sustain the inflammatory process by recruiting monocytes, immature DCs, and other inflammatory cells to the site of antigen. At later time points, when mature DCs reach T cell areas of lymphoid tissue, they produce high levels of lymphoid chemokines such as ELC, macrophage-derived chemokine (MDC), thymus and activation-regulated chemokine (TARC), pulmonary and activation-regulated chemokine (PARC), and IFN- $\gamma$ -inducible protein (IP-10) (247, 288, 346). The production of lymphoid chemokines by mature DCs in lymphoid tissue recruits T cells, augmenting the chances of DC-T cell contact. As previously discussed, ELC binds CCR7 expressed on naive T and B cells and mature DCs. MDC and TARC bind CCR4, a receptor expressed on recently activated T cells, but not on naive T cells (73, 282). PARC binds to an unidentified receptor expressed on naive T cells (3). IP-10 is a chemoattractant that mediates the migration of plasmacytoid DCs directly from blood to the inflamed lymphoid tissue. Plasmacytoid DCs express the IP-10 ligand CXCR3 that can also bind to monokine induced by IFN- $\gamma$  (Mig) produced by mature DCs.

Consolidating what we know about the unique migratory patterns of myeloid and plasmacytoid DCs and their patterns of IL-12 production, Patterson (255) has proposed a model delineating the contributions of myeloid and plasmacytoid DCs to the generation and regulation of an immune response to a viral infection. Myeloid precursor DCs leave the blood and home to various tissues in response to chemoattractant gradients. Immature myeloid DCs are located at sites where most pathogens enter the body. Upon exposure to virus, immature myeloid DCs bind and internalize virus and subsequently release inflammatory cytokines and chemokines that initiate the recruitment of more immature myeloid DCs and other leukocytes to the site of infection. Maturing DCs carrying antigen migrate via afferent lymphatics to T cell areas of draining lymph nodes, where they interact with and stimulate pathogen-specific T cells. Myeloid DCs initially release IL-12 and drive the generation of effector cells with a Th1 phenotype. However, within 24 h, production of IL-12 ceases and IL-4-secreting T cells are generated to dampen the Th1 response (53). Coordinately, a switch away from the generation of effectors to unpolarized memory T cells occurs. Myeloid DCs in lymph nodes produce chemokines that recruit T cells, B cells, more myeloid DCs, and plasmacytoid DCs. In response to IP-10 and Mig, plasmacytoid DCs leave the blood and enter inflamed lymph nodes through HEV. The expression of CD62L on plasmacytoid DCs allows them to migrate via HEVs (186). In lymph nodes, plasmacytoid DCs are stimulated via the expression of CD40L on recently activated T cells to mature and secrete IL-12 and maintain production of IFN- $\alpha$  (53). The

production of IFN- $\alpha$  by plasmacytoid DCs prolongs the Th1-stimulating phase of myeloid DCs. The unique migratory properties and controlled temporal release of IL-12 by myeloid and plasmacytoid DCs provides the generation of the right kind of T helper cell at the right time.

#### IV. DENDRITIC CELL-T CELL INTERACTIONS

During the development of an adaptive immune response, the phenotype and function of DCs play an important role in initiating tolerance, memory, and polarized Th1 and Th2 differentiation. As discussed, DC subsets have been proposed as playing differing roles in defining the outcome of an immune response, although clearly some plasticity within defined subsets is possible so that each subset can exert tolerizing and polarizing influences on responding T cells (255). Important factors other than signals delivered by DCs that drive primary immune responses are concentration of antigen in the microenvironment, concentration of cytokines and other soluble factors present in the fluid phase in the vicinity of the APC-T cell interface and, of course, the genetics of the host that may limit how the interacting cells may respond. For example, studies in which APCs are replaced by molecular complexes on plastic or lipid substrates have demonstrated that T cells can be polarized by adding cytokines to the culture systems. Still, delivery of the relevant signals by DCs at the DC-T cell interface is likely the most efficient and physiologically relevant mechanism for initiating an immune response.

CD4 and CD8 T cells respond to peptide antigen displayed on MHC class II and MHC class I molecules, respectively (referred to as *signal 1*, see Fig. 3). Accessory molecules on DCs are required to ensure that T cells will divide and differentiate into effector cells (*signal 2*, Fig. 3). In the absence of sufficient costimulation, T cells exhibit anergy or undergo apoptosis. Secretion or lack of secretion of factors by DCs, particularly IL-12, are instrumental in the final differentiation of T cells into type 1 or type 2 effector T cells, respectively (*signal 3*, Fig. 3). The model illustrated in Figure 3 is the simplest one to explain the development of a productive immune response by CD4 T cells, but the list of membrane and secreted molecules that play roles in regulating the interaction of DCs and T cells is growing.

##### A. Antigen Uptake

Initially, DCs were described as both nonphagocytic and nonendocytic. Macrophages are avidly phagocytic, but the antigens taken up are rapidly degraded, unless the material that is endocytosed is inert or is a microorganism with the ability to prevent phagosomal-lysosomal fusion and/or enzymatic degradation. Thus the absence of macrophage phagocytic mechanisms was troublesome for explaining the ability of DCs to take up and present peptides from complex antigens. We now know that immature DCs are avidity en-

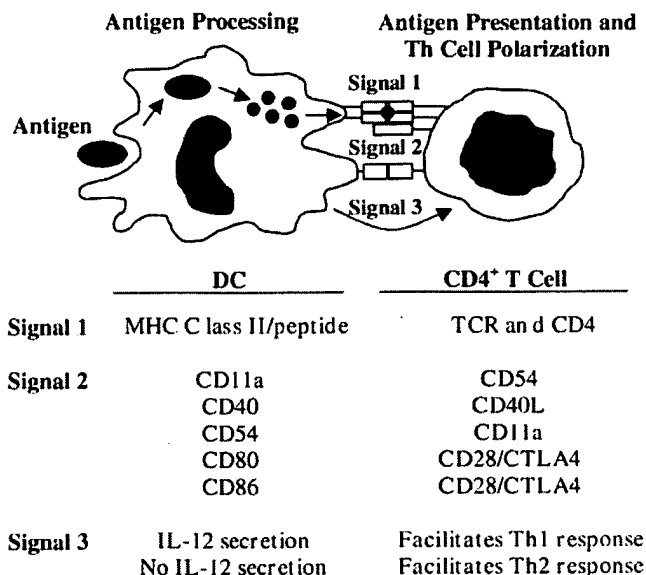


FIG. 3. Model for DC-CD4<sup>+</sup> T cell interaction. DCs provide three signals to antigen-specific CD4<sup>+</sup> T cells to initiate T cell proliferation and differentiation. DCs take up antigen and readily degrade antigens to produce antigenic peptides capable of binding to MHC class II. DCs express a high density of MHC class II/peptide complexes on their cell surface for recognition by the T cell receptor (TCR) expressed on CD4<sup>+</sup> T cells (*signal 1*) and costimulatory molecules (*signal 2*) to stimulate CD4<sup>+</sup> T cell proliferation. Secretion or lack of secretion of interleukin-12 (IL-12) by DCs (*signal 3*) is important in the final differentiation of CD4<sup>+</sup> T cells into type 1 or type 2 effector T cells, respectively. All accessory molecules are not included. See text for additional details.

docytic, whereas mature DCs have downregulated this activity (330). DCs degrade antigens within a MHC class II-rich endosomal compartment (MIIC) yet preserve sufficient peptide structure to be expressed on their cell surface bound to MHC class II molecules. DCs take up antigens by phagocytosis, utilizing membrane receptors to trigger uptake, by receptor-mediated pinocytosis in clathrin-coated pits and by fluid-phase pinocytosis. DCs can take up whole cells, including necrotic and apoptotic cells. They can also acquire antigens from live cells for presentation to cytolytic T cells (133). Receptors available to some or all DC subsets for antigen uptake include the Fc $\gamma$ Rs CD32 and CD64; the high- and low-affinity IgE receptors Fc $\epsilon$ RI and Fc $\epsilon$ RII (CD23), respectively; the complement receptors CD11b and CD11c; a C lectin type of mannan binding receptor, DEC205 (CD205), and the scavenger receptor pair for apoptotic cells  $\alpha_v\beta_5$  and CD36 (reviewed in Refs. 134, 191). During maturation, as endocytosis decreases, these receptors are usually downregulated. More recently, immature human interstitial DCs were shown to express the Fc $\alpha$ R CD89 (117).

##### B. Antigen Processing

###### 1. MHC class II presentation

Antigen processing by DCs occurs primarily through two major pathways: an exogenous or endosomal path-

way and an endogenous or proteosomal pathway (Fig. 4). Exogenous antigens gain access to early and late acidic endosomal compartments in which proteases initiate degradation. The peptide fragments then associate with pre-formed MHC class II molecules within the MIIC. MHC class II  $\alpha$ - and  $\beta$ -peptide chains are synthesized in the endoplasmic reticulum (ER), where they associate with invariant chain (Ii) (46). Ii protects the peptide-binding groove of the MHC class II heterodimer from being prematurely filled with self-proteins. The MHC class II/Ii complex is then transported from the ER through the Golgi from which vesicles deliver the complexes to the MIIC. There partial proteolytic cleavage of Ii occurs leaving a small fragment called CLIP (class II-associated invariant-chain peptide) in the peptide-binding groove of the MHC class II molecule (302). Another molecule, HLA-DM in humans or H-2M in the mouse, which have a structure similar to the MHC class II molecules, removes MHC class II-associated CLIP. This step allows endosomal antigenic peptide to take its place (241). Finally, MHC class II with the new antigenic peptide in its binding groove traverses the cytoplasm in exocytic vacuoles for display on the cell surface. Surface MHC class II molecules can be recycled from the cell surface through en-

docytic pathways and acquire new antigens in the MIIC. Immature DCs accumulate MHC class II and degraded peptide in lysosomal vesicles until the DCs are activated. After DC activation, the MHC class II/peptide complexes accumulate in nonlysosomal vesicles that migrate to the cell surface. It has recently been demonstrated that members of the B7 family of costimulatory molecules are embedded in the vesicular lipid along with MHC class II and are delivered to the cell surface in association with the MHC class II/peptide complexes (359).

## 2. MHC class I presentation

For MHC class I to display peptide antigens to CD8 T cells, DCs degrade cytoplasmic proteins in proteasomes and likely with the help of cytosolic heat shock proteins (HSPs) acting as chaperones, transport the resultant peptides via the heterodimeric transporter associated with antigen processing (TAP) into the ER (see Fig. 4, Ref. 254). In the ER, newly synthesized MHC class I  $\alpha$  chains form complexes with  $\beta_2$ -microglobulin ( $\beta_2$ M) that then bind the resulting peptide antigens. Within the ER, chaperone proteins, including calnexin, calreticulin, and HSP gp96, aid in peptide binding and proper folding of the

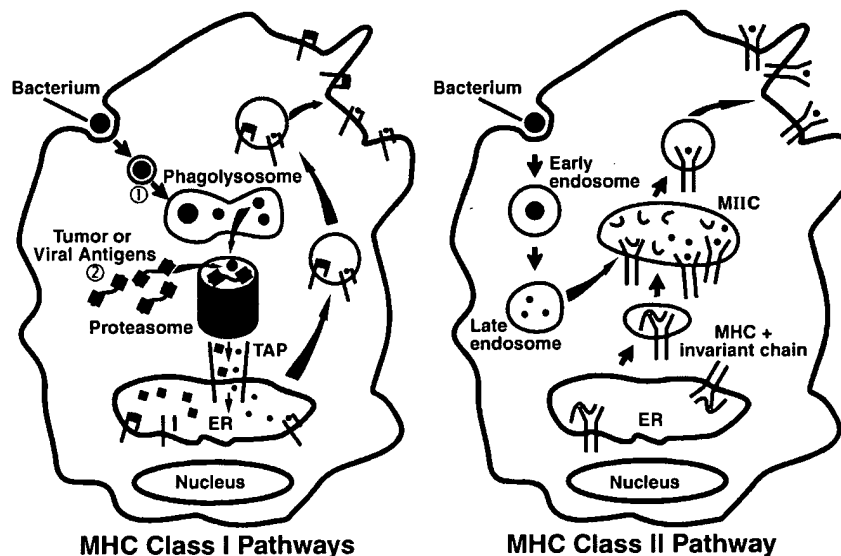


FIG. 4. DC antigen processing. The major MHC class I pathways are depicted on the *left*. 1) Antigen is taken up by phagocytosis and receptor-mediated endocytosis, undergoes limited proteolysis, and by active transport enters the cytosol. The cytosolic antigens are further degraded via the proteosomal pathway, enter the endoplasmic reticulum (ER) utilizing TAP, and are bound to newly synthesized MHC class I molecules. MHC class I/peptide is subsequently carried by vesicular transport to the cell surface. 2) Endogenous proteins are similarly degraded via the proteosomal pathway, enter the ER utilizing TAP, are bound to MHC class I, and by vesicular transport reach the cell surface. The major MHC class II pathway is shown on the *right*. Antigen is taken up by phagocytosis, receptor-mediated endocytosis, and fluid-phase pinocytosis through the early and late endosomes, during which time some proteolysis occurs. The peptides enter the MHC class II-rich vesicular compartment (MIIC) where they are bound in the MHC class II peptide-binding groove and are then transported to the cell surface. MHC class II is synthesized in the ER where invariant chain (Ii) protects the groove from premature binding of self-peptides. Ii is further degraded into a smaller peptide (called CLIP and shown as a fragment in one of the three MHC molecules in MIIC) to ready itself for its replacement by the antigenic peptide in MIIC. See text for additional details.

MHC class I/ $\beta_2$ M complexes. Then tapasin facilitates the formation of the MHC class I/ $\beta_2$ M/peptide complexes (25, 273). Finally, MHC class I/ $\beta_2$ M/peptide complexes are transported in exocytic vesicles to the DC plasma membrane.

It was originally thought that the proteosomal pathway processed only those proteins synthesized within APCs. It is now known that antigens can escape by poorly understood mechanisms from endocytic pathways, undergo proteasome-dependent degradation, and subsequently enter the ER via the TAP pathway to be presented in the binding groove of MHC class I molecules as is true for endogenous proteins (258, 270). This process is referred to as cross-presentation, and the resulting primary immune response is referred to as cross-priming (Fig. 4). The antigens may be particulate or soluble antigens, or come from dead and dying cells or from exosomes, which are small vesicles pinched off from the membranes of immature DCs and contain MHC molecules (191, 395). Cross-priming can lead to both productive immunity and tolerance of CD8 T cells. Cross-priming is the mechanism by which DC process neoplastic or infected self cells as well as engrafted allogeneic cells (191). DCs are the cells that are fully sufficient to perform cross-priming (184). Furthermore, at least in one in vivo murine study, this capacity seems restricted to the splenic CD8 $\alpha^+$  population even though the CD8 $\alpha^-$  population was also capable of taking up the antigen (81). In any case, antigen uptake is an immature DC process and requires activation of the APC by costimulatory activation signals, such as cross-linking CD40, before cross-priming of the responder CD8 T cells can effectively occur. It has been postulated, and we shall return to this, that uptake of apoptotic cells by DC leads to tolerance induction, whereas DC uptake of necrotic cells tends to activate DCs to induce cytolytic CD8 T cells.

### 3. CD1 presentation

DCs are also capable of presenting antigens on CD1 molecules. CD1 molecules are a family of nonpolymorphic histocompatibility antigens associated, like MHC class I molecules, with  $\beta_2$ M (38, 303). CD1 molecules are present on myeloid DCs and, indeed, CD1a has frequently served as a marker for identifying these cells. Five CD1 isoforms, CD1a-e, have been described in humans, but only two CD1 homologs, CD1d1 and CD1d2, are expressed in the mouse and rat. The murine CD1d1 molecule crystal structure shows that it is constructed to bind very hydrophobic ligands, compatible with their presenting antigenic lipids (391). CD1 molecules present lipid and glycolipid antigens of both endogenous and exogenous derivation. The role of CD1d as a restricting element for endogenous lipid was shown to be relevant for self-antigen recognition by natural killer (NK)1.1+ T cells in mice

(28). These  $\alpha/\beta$  T cells have a restricted receptor binding repertoire in mice comprised of a V $\alpha$ 14-J $\alpha$ 281 TCR  $\alpha$ -chain paired with a restricted set of  $\beta$ -chains, and in humans comprised of V $\alpha$ 24-J  $\alpha$ Q/V $\beta$ 11. Upon responding to CD1/lipid antigen complexes, NK1.1+ T cells produce IL-4 or IFN- $\gamma$  and have been implicated in recognition of infectious agents, tumors, and autoantigens. NK1.1+ T cells may express CD4 molecules or lack both CD4 and CD8 (28), and their cytokine production may be regulated by *signal 3*, i.e., IL-12 released by the CD1+ DCs (387). A second T cell subset restricted by CD1d on APCs has also been identified that does not express NK1.1. This T cell subset has a limited TCR repertoire and, like NK1.1+ T cells, can release either IL-4 or IFN- $\gamma$  or express cytotoxicity (60, 370). Less is known about the TCR repertoire and its restriction to CD1a-c associated lipids in humans, but it is clear T cells can be restricted by these molecules, demonstrate limited diversity, and may bear either the  $\alpha/\beta$  or  $\gamma/\delta$  TCR (303).

Antigen processing and presentation by CD1 is different from that described above for MHC class I and II. CD1 molecules are synthesized in the ER and are expressed on the plasma membrane following traffic to the surface via vesicular transport. CD1 molecules are subsequently incorporated into endosomes and become associated with lipid ligands and recycle to the plasma membrane. Different CD1 homologs may associate with antigens in distinct endosomal compartments (337). In human myeloid DCs, CD1b binds to lipids that have been degraded in the deep endosomal compartments, whereas CD1a and CD1c associate with their corresponding lipid antigens in the recycling vesicular compartments of the early endocytic system. The purpose for the maintenance of these nonpolymorphic restricting elements during evolution may be to allow surveillance of normal intracellular lipid pathways, rather than for the development of protective immunity (303). However, it has been speculated that some microbes, such as *Mycobacterium tuberculosis*, developed the capacity to usurp CD1 molecules for their own purposes by having mycobacterial lipids presented to responder T cells to generate a granulomatous and necrotizing inflammatory response in the host. This process may allow the microbe to avoid complete eradication until it can be transmitted to the next host.

## C. Costimulation

### 1. B7 family

Costimulation is required to initiate productive immune responses by T cells. The first and most important costimulatory molecules characterized were CD28 on naive T cells and the corresponding ligands, CD80 (B7-1) and CD86 (B7-2), which are upregulated on maturing APCs (69, 297). CD80 and CD86 molecules were the first

described members of what is now known to be as a larger B7 subfamily; members of this subfamily belong to the immunoglobulin superfamily of proteins (see Table 2). CTLA-4 was later identified with strong homology to CD28 and is upregulated on activated T cells, binds with a higher affinity to CD80 and CD86 than CD28, and down-regulates the immune response (369).

A third B7 family member is B7RP-1. B7RP-1 is expressed predominantly on B cells but is also found on macrophages, DCs, and nonlymphoid tissue cells and is the ligand for the inducible immune costimulator (ICOS) protein (175, 341, 389). ICOS is structurally related to CD28 and, like CTLA4, is upregulated on activated T cells (147). ICOS knockout mice demonstrate severely deficient T cell-dependent Th cell responses in which both the Th2-dependent antibody isotype IgG1 and the Th1-dependent antibody isotype IgG2a are decreased in the serum (88, 232, 343). Immune responses in ICOS knockout mice were characterized as defective with an absence of germinal center formation, a marked impairment of T cell IL-4 secretion, and IgE isotype switching. However, ICOS knockout mice were able to mount a Th1 cellular response, as noted by antigen-induced T cell IFN- $\gamma$  secretion in response to immunization. The impaired T cell-dependent B cell responses suggest that after initial DC-T cell interactions in the paracortex of secondary lymphoid tissue, an important ICOS-dependent T cell-B cell interaction occurs that may finalize T cell subset differentiation. Importantly, in one study, addition of CD40 to upregulate CD40L largely repaired the ICOS knockout defect, suggesting the role of ICOS interacting with its ligand B7RP-1 on APCs is to facilitate the downstream CD40L-CD40

interaction, an interaction already known to be required for T cell-dependent B cell responses (232). The observation that in ICOS knockout mice experimental allergic encephalitis was exacerbated (88) suggested that peripheral antigen presentation by nonprofessional APCs might be important during the effector limb of immune-mediated inflammation. This suggestion is supported by the observation that the ICOS ligand B7RP-1 appears on non-lymphoid tissues. A clear role for B7RP-1 on DCs has not yet been established.

A fourth B7 family member, PD-L1 (also called B7-H1), is constitutively expressed on DCs and binds to the programmed cell death 1 (PD-1) receptor on T cells (reviewed in Ref. 69). This interaction exerts an inhibitor function on T cell proliferation and cytokine production. A related B7 family member, PD-L2, plays a similar role in inhibition and also binds to PD-1 but seems to have a more important role in Th2 inflammatory states, whereas PD-L1 participates in Th1 inflammation (69). Finally, B7-H3 has been recently identified and cloned (58). It is highly expressed on immature DCs and is downregulated on mature DCs, in contrast to the expression of most of the other B7 family molecules. Although the ligand for B7-H3 is unknown, it is not CD28, CTLA4, ICOS, or PD-1. B7-H3 plays an enhancing role in costimulation of both CD4 and CD8 T cells and in the induction of IFN- $\gamma$  production, another surprising function in view of its higher expression on immature DCs.

## 2. TNF family

The TNF family of ligands and receptors, now totaling ~50, are also important costimulators in DC interactions with T cells (Table 2). Particularly noteworthy is the interaction of CD40 on DCs with CD40L (CD154) on T cells. CD40 was first identified as a critical B cell molecule that interacted with CD40L on T cells to allow for isotype switching. During effective DC-T cell interactions, as T cells become activated, they upregulate CD40L. CD40L can then interact with CD40 on mature DCs to trigger IL-12 release, required for Th1 polarization (50, 56). Additional TNF family members, OX40 on T cells and OX40L on DCs, also play an important complementary role for inducing T cell proliferation and cytokine production (372). OX40L knockout mice fail to generate contact hypersensitivity (59), and OX40 knockout mice demonstrate reduced CD4 T cell proliferation, IFN- $\gamma$  production, and protection against an influenza lung infection (176). Both OX40 and OX40L knockout mice demonstrate no humoral immune response defects, and OX40 knockout mice retain primary and memory cytotoxic T cell responses. Other TNF family members listed in Table 2 have been shown to provide important signals that enhance CD8 T cell proliferation and IFN- $\gamma$  production (4-1BBL on DCs and 4-1BB on T cells) and DC cytokine secretion and

TABLE 2. Costimulation molecules for DC-T cell signals

DC	T Cell
<i>B7 family and receptors</i>	
B7-1 (CD80)	CD28, CTLA-4 (CD152)
B7-2 (CD86)	CD28, CTLA-4 (CD152)
B7RP-1	ICOS
PD-L1 (B7-H1)	PD-1
PD-L2	PD-1
B7-H3	Not known
<i>TNF family ligand and receptors</i>	
CD40	CD40L (CD154)
OX40L	OX40 (CD134)
4-1BBL	4-1BB (CD137)
TRANCE (RANK)	TRANCE (RANK-L)
CD27	CD27L (CD70)
CD30L (CD153)	CD30
<i>Miscellaneous</i>	
ICAM-1 (CD54)	LFA-1 (CD11a/CD18)
DC-SIGN (CD209)	ICAM-3 (CD50), ICAM-2 (CD102)
SLAM* (CD150)	SLAM (CD150)
CD58	CD2

TNF, tumor necrosis factor; ICAM, intracellular adhesion molecule. \* SLAM binds to itself.

survival RANK on DCs and RANK-L on T cells (reviewed in Ref. 21).

### 3. Other costimulatory molecules

Finally, a heterogeneous group of receptors has been described as regulating DC-T cell interactions (Table 2). The leukocyte function antigen-1 (LFA-1; or CD11a/CD18) interaction with ICAM-1 (CD54) induces an adhesive interaction between DC and T cells which when disrupted markedly reduces the proliferative response of T cells stimulated by DCs (19, 229). However, this molecular interaction might also influence the type of immune response that develops in the T cell. In experiments using T cells from DO11.10 mice that express a TCR specific to OVA peptide 323–339 and stimulated by OVA peptide on splenic DCs, it was shown that interactions of LFA-1 with intracellular adhesion molecule (ICAM)-1 and ICAM-2 were important for generating Th1 responses. Thus blocking the interaction with a combination of anti-ICAM-1 and anti-ICAM-2 shifted the *in vitro* Th1 immune response to a Th2 response (290). DC-SIGN (which comes from DC-specific, ICAM-3 grabbing nonintegrin) is a 44-kDa type I membrane protein with an external mannose-binding, C-type lectin domain (324). It has been postulated that the interaction of DC-SIGN on DCs with ICAM-3 on T cells is required to initiate effective interaction of the MHC class II/peptide complex on DCs with the TCR on T cell (115). The postulate is that the DC-SIGN-ICAM-3 interaction allows sufficient DC-T cell adhesion for signaling to occur. DC-SIGN also binds to ICAM-2, and this interaction seems important for DC migration across both resting and activated endothelium (116).

### D. Events at the DC-T Cell Interface

A signal from an APC can be transmitted to a recently activated T cell rather quickly, whereas signaling a naive T cell may require more prolonged interaction. Time lapse microscopy has enabled the study of T cells interacting with planar membranes in which fluorescence-labeled adhesion molecules and MHC-peptide complexes can freely diffuse. Thus the planar membrane mimics to some extent the activity of antigen-pulsed APCs. With the use of this system, early central aggregation of LFA-1 with ICAM-1 (within 30 s) can be observed followed within 20 min by aggregation of the MHC/peptide complexes by the TCR, constituting *signal 1*. The clustering of complexes at the DC-T cell interface has been referred to as the “immunological synapse” (reviewed in Ref. 124). In addition to surface molecules, signaling molecules in the T cell such as Lck, Fyn, and ZAP 70 have been observed in the immunological synapse. Both the clustering of DCs with T cells as well as the subsequent T cell proliferative response is dependent on the reorganization of the T cell

actin cytoskeleton and is characterized by the accumulation of filamentous actin and other cytoskeletal proteins at the T cell-DC interface. It was recently shown that disruption of the cytoskeleton by cytochalasin D in DCs interfered with effective clustering and activation of responder T cells (6). Prolonged binding of T cells to DCs has been shown in some systems to be required for optimal antigen stimulation (22). Others have noted that the time for DC-T cell interaction determines the immunological outcome with shorter periods of interaction favoring anergy and death, intermediate periods the development of memory, and longer periods resulting in differentiation into effector cells (187, 189). In a recent study in which DCs were allowed to interact with T cells in collagen gels, T cells were observed to crawl continuously over the surface of DC surface in short 6- to 12-min encounters. The repeated encounters resulted in T cell calcium influx and activation as measured by increases in activation markers and finally proliferation (131). This study raises the question as to whether the T cell needs to remain in an immobile state adhered to a DC to develop into a memory or effector T cell as implied by studies on planar members or whether, in contrast, active migration is the norm. In the intact animal, after subcutaneous antigen inoculation, initial interactions occur between DCs and T cells in T cell areas and continue at the interface of the T cell area with the follicles (153). In these latter *in vivo* studies, whether the DC-T cell interactions that resulted in expansion of the T cell population reflected prolonged immobilization of T cells by DCs or whether repeated short-lived interactions occurred could not be assessed.

## V. ROLE OF DENDRITIC CELLS IN LINKING INNATE AND ACQUIRED IMMUNITY

### A. Microenvironment Influences DC Phenotype and Function: Recognizing Danger

DCs are members of the innate immune system, deployed throughout the body to sample the environment and determine whether a host response is needed and, if so, what kind of response. Infectious agents pose a threat to the normal host, who is generally well-equipped to resist these attacks. On infectious challenge of the non-immune host, innate immunity expresses itself quickly with appropriate defenses, often in the absence of a detectible inflammatory response. An example of a regional innate response is mucociliary clearance in the lung, which daily exerts effective antimicrobial protection. Nevertheless, the ability of the lung to also respond with an adaptive immune response is essential, as has been learned from examples of multiple opportunistic pulmonary infections in victims of the human immunodeficiency virus (HIV). In 1992, Polly Matzinger proposed that it was

the innate immune system that recognized danger and delivered nonspecific signals to specific T and B cells, stimulating them as to clonally divide and differentiate into effector lymphocytes and antibody-secreting cells, respectively (231, 234). Thus the older idea that the immune system learns a complicated set of rules early in ontogeny about how to recognize self and respond only to "not self" was exchanged for a simpler model. The newer model is that professional APCs respond to the environment and carry an antigenic message to responder T cells to instruct them to either develop tolerance or productive immunity. Current experimental evidence supports this notion. If a foreign or self-antigen is not dangerous, tolerance is the expected outcome, because DCs will not mature and, therefore, will not deliver a second signal. A corollary is that if a danger signal is given when self-antigens are processed, then an autoimmune response might develop.

As the initiator of T cell responses, DCs respond to danger signals and modify their function to generate an adaptive immune response. They use receptors to respond to the environment, first to take up, process, and present antigens and also to receive the danger signal. The "danger signal" receptors activate DCs causing them to engage intracellular machinery that 1) allows MHC class II to associate with immunogenic peptides in the proper endosomal compartment, 2) facilitates accumulation of MHC class II/peptide complexes in vacuoles together with costimulatory molecules that are subsequently coexpressed in domains on the cells surface, and 3) releases cytokines that further modulate the immune response (152, 166, 359).

Danger signals may arise from endogenous pro-

cesses, particularly those that result in cell necrosis and tissue destruction (Table 3). Examples of specific signals of this type are cellular HSPs, matrix degradation products such as hyaluronan, and cellular cytokines and cell surface ligands such as TNF- $\alpha$ , IL-1, and CD40L (4, 347). The normal turnover of cells is accomplished through programmed senescence whereby cells die via apoptosis, and neither inflammation nor development of adaptive immunity is a desirable outcome. On the other hand, when tissue is injured, particularly when necrosis results, inflammation is expected to initiate an appropriate repair response. HSPs are particularly important in signaling the host that something is amiss. Apoptotic cells do not express HSPs to stimulate an immune response. In contrast, before or as they die, necrotic cells release HSPs into the microenvironment (24). HSPs bind to DC receptors, induce DC maturation, and stimulate migration of DCs into secondary lymphoid tissue. The endogenous HSPs, HSP70, HSP90, and HSP96, all bind to CD91, which is present on DCs (23, 29, 30, 309).

Danger signals also derive from foreign substances. Microorganisms display motifs referred to as pathogen-associated molecular patterns (PAMPs) that stimulate DCs to undergo maturation. Microbial products are among the most common exogenous danger signals, and specific components include LPS from gram-negative organisms, peptidoglycans, and lipoteichoic acids from gram-positive organisms, microbial DNA which is rich in CpG motifs, microbial HSPs, and double-stranded viral RNA (see Table 3, Refs. 4, 14, 112). The most important receptors on DCs that recognize microbial products and transmit the message to initiate adaptive immunity are the toll-like receptors (TLRs), a family of highly conserved

TABLE 3. *Molecules that act on DCs to mediate the link between innate and acquired immunity*

Endogenous Danger Signals for DC (Their Known Receptors)	Exogenous Danger Signals for DCs (Their Known Receptors)
Necrotic cells (24, 25, 111)	Bacteria and bacterial products
Stress-induced release of intracellular ATP and UTP (purinergic receptor P <sub>2Z</sub> /P <sub>2X7</sub> ) (244)	Peptidoglycans, lipoteichoic acids, lipopeptides (TLR-2)
Reactive oxygen intermediates (281)	LPS (TLR-4)
Matrix degradation products, such as hyaluronan (possibly CD44) (347)	CpG (TLR-9) (14)
Endogenous heat shock proteins	Mycobacteria
HSP70, HSP90, and HSP96 (CD91) (23)	Lactobacilli
HSP60 (TLR-4) (250)	<i>Leptospira interrogans</i> (TLR-2) (375)
CD40L (CD40)	Viruses
Cytokines	Herpes simplex
TNF- $\alpha$ (TNF- $\alpha$ -R)	Influenza virus and dsRNA (55)
IL-1 $\beta$ (IL-1 $\beta$ R)	Dengue virus (140)
Type I IFNs (111, 165)	Viral mRNA (373)
Vasoactive intestinal polypeptide (77)	Fungi
Aggregated IgG (Fc $\gamma$ Rs)	Mannans
Fas (FasL) (274)	<i>Candida albicans</i> (90)
	<i>Histoplasma capsulatum</i> (VLA-5) (119)
	Parasites
	<i>Acanthocheilonema vitae</i> (376)
	<i>Leishmania major</i> (224)
	<i>Toxoplasma gondii</i> (335)

Danger signals not specifically referenced as shown in parentheses are discussed and referenced in 4, 112, 120, 231, 234. Reference numbers are given in second set of parentheses. TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; IFN, interferon; LPS, lipopolysaccharide.

molecules initially described in *Drosophila*. TLRs are type I integral membrane receptors with extracellular leucine-rich regions and intracellular domains homologous to the signaling domain of IL-1R. Specific microbial ligands have been identified for vertebrate TLRs 2, 4, and 9 (see Table 3, Refs. 4, 135, 349). Upon interaction with their agonists, TLRs 2, 4, and 9 signal through the myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinase (IRAK), TNF receptor-associated factor (TRAF), and the NF- $\kappa$ B/Rel proteins, which finally results in NF- $\kappa$ B translocation to the nucleus and transcriptional activation (10, 166). In a study examining the expression of TLRs 1–5 on human leukocytes, human DCs expressed all five, and TLR 3 was exclusively expressed on DCs (245). TLR 1–5 mRNAs were downregulated during the maturation of DCs. Furthermore, DCs responded to LPS via TLR 4, secreting TNF- $\alpha$  but only while they were immature (367). These results suggested that only immature DCs are fully capable of responding to microbial products. Thus, after DCs mature, they would have only a short-lived ability to influence the naive T cell within secondary lymphoid organs. Thereafter, DCs would become unable to secrete polarizing cytokines such as IL-12 and undergo senescence, limiting the time frame within which they could stimulate immunity. In summary, DCs link innate and adaptive immunity by receiving danger signals that render them capable of maturing and inducing productive immunity rather than tolerance.

## B. DC Role in T Cell Memory, Effector Function, and Tolerance

DCs regulate primary immune responses by directing antigen-specific T cells to die or to become anergic, memory, or effector T cells. In addition, the cytokines synthesized or the lytic machinery generated define the type of effector T cell, i.e., Th1, Th2, cytotoxic, or regulatory T cell. Lanzavecchia and Sallusto (188) have proposed a linear differentiation model for T cells during priming based on the persistence of the DC-T cell interaction in lymphoid organs. According to the model, the length of time that T cells and DCs interact defines effector function, homing, and survival of responder T cells with only the fittest T cells maintaining a DC-T cell interaction and surviving to become memory T cells. Excessive stimulation causes responding naive T cells to proliferate and develop effector function, but many of these responders soon die (157), some within the lymphoid organ and others after they migrate to tissue sites. Some effector T cells survive and persist as effector memory cells as the primary response wanes, although the survival signals these cells receive are not certain. Inadequate stimulation, either through a poor fit of the TCR for the DC's MHC/peptide complex (159, 310) or lack of costimulation

as a result of a low level of DC costimulatory molecules or both, would lead to T cell anergy or programmed cell death. Finally, as the model continues, a second type of memory T cell, a central memory T cell, develops if the strength of DC stimulation falls somewhere intermediate between that required for generating anergy and that required for polarizing effector T cells (285). Both types of memory T lymphocytes survive for years ready to respond to cognate antigen displayed on APCs. Central memory T cells circulate between blood and lymphoid organs and respond to MHC/peptide complexes by further expansion and differentiation into effector cells, which subsequently migrate to relevant target sites. In contrast, effector memory T cells circulate between blood and peripheral tissues and, thus, can quickly respond to antigen by displaying immediate effector function (285). Each memory T cell type has distinct markers that allow them to migrate to the appropriate tissue site and to be distinguished from naive and effector T cells generated directly in the primary immune response.

### 1. Generation of memory

Memory cells may be preserved through the persistence of antigen, but antigen is not required for persistent CD8 T cell memory (192). In one study, memory CD8 T cells were shown to persist, depending on the balance of IL-15 with IL-2 (181). DCs secrete IL-15 (32, 161, 163), which could contribute to persistence of memory CD8 T cells; however, to date the role for DCs, if any, in persistence of CD8 T cell memory is incompletely understood. Mechanisms for maintenance of CD4 T cell memory are currently a focus for study. Van Essen et al. (363) demonstrated that CD4 T cell memory depended on DCs to process and present antigen. The source of the antigen was thought to be antigen-antibody complexes on follicular dendritic cells (FDCs). This study also demonstrated a requirement for B cells to maintain memory development, likely to facilitate the development of FDCs and to secrete the complex-forming antibody.

### 2. Effector T cell generation

Effector T cell differentiation from naive T cells requires prolonged TCR contact with MHC class II/peptide complexes and costimulation to induce proliferation and cytokine secretion to finally polarize the T cells (148, 188). DCs are ideal cells for performing this function, because expression of MHC class II/peptide complexes persist on mature DCs for over 100 h (52). T cells with high avidity interactions with DCs are the most likely to successfully compete for a long-term interaction with antigen-bearing APCs and, therefore, to repeatedly divide yielding daughter T cells capable of polarizing into effector cells. DCs that secrete IL-12 induce Th1 polarization (137), but, as has already been discussed, DCs are capable of producing

IL-12 for only a short time (186). After 8–12 h, DCs exhaust their ability to produce IL-12 and subsequently activate proliferating T cells toward either a Th2 response or a regulatory T cell response. Interestingly, stimulation of Th1 subset polarization requires less time for the DC-T cells interaction than Th2 subset polarization, because there is an additional time requirement to induce demethylation of the IL-4 and IL-13 genes that is required for Th2 cell generation (149). If DCs emigrate into lymphoid tissue incapable of making IL-12, continued stimulation of naive T cells will induce the Th2 subset. In the absence of IL-12, the ability of responding naive T cells to make small amounts of IL-4 favors the development of Th2 T cells by autocrine stimulation. Most pathogens induce DC IL-12 synthesis and secretion while other factors such as PGE<sub>2</sub>, IL-10, and selected microbes, such as hyphal forms of *Candida albicans*, inhibit IL-12 secretion (90; see Table 4 for a list of IL-12 inducing and suppressing stimuli). Because of the transient IL-12 production by antigen-presenting DCs, the continual influx into the regional responding lymphoid organs of fresh IL-12-secreting DCs from the peripheral site is required to drive a large pool of responders to become Th1 cells. Alternatively, as the antigen-specific T cells become activated, their expression of CD40L may also continue to enhance IL-12 secretion by DCs to levels needed to maintain a Th1 response. However, a CD40L signal is not successful in driving DCs to make IL-12 unless the DCs received a prior microbial signal (138, 313). As discussed, plasmacytoid DCs that carry antigens from systemic infected sites may also directly enter the T cell area of the lymph node by HEVs and continue to drive a Th1 response. In any case, once an infection clears and the microbial stimulus is gone, the ability for DCs to continue to make IL-12 would not be expected to continue.

### 3. Tolerance

Tolerance is the specific inability of a host to respond to antigens and is generated both centrally and peripher-

ally. Central tolerance mechanisms occur in the thymus for T cells and in the bone marrow for B cells (1). T cells that might inadvertently respond to DCs carrying self-peptides are deleted during ontogeny in the thymus. T cells that fail to respond to stimuli in the thymus die from neglect, while T cells that recognize MHC/peptides with high avidity undergo apoptosis and are deleted; this latter process is called negative selection. T cells that recognize self with low avidity in the thymus survive, a process called positive selection, and reach the periphery where they respond only to antigens presented in the context of self-MHC. Thymic epithelial cells are responsible for presenting self-peptides in the context of MHC for positive selection. Both thymic DCs and thymic epithelial cells contribute to negative selection (39, 40, 394).

### 4. Peripheral tolerance mechanisms

Peripheral tolerance mechanisms include T cell death, T cell anergy, and active suppression by T regulatory cells. Once in the periphery, as described above, productive immune responses occur when DCs that have taken up antigen are activated and present optimal levels of MHC/peptide complexes in the context of accessory molecules. In the normal host if self-antigens are presented, no T cells should be available to respond, because of central tolerance induction. However, if T cells recognize only low levels of MHC/peptide, have a low affinity for their cognate ligand, or receive no costimulation from DCs, they become anergic or undergo apoptosis. For example, immature DCs treated with IL-10 fail to mature and, as a result, induce anergy in responder T cells (323). Once generated, anergic T cells can suppress development of an immune response by directly suppressing the expression of MHC class II, CD80, and CD86 on DCs in culture (365).

Tolerance can also be achieved by the induction of T regulatory cells. Th0, Th1, and Th2 T cells can be suppressed by T regulatory cells that secrete suppressive factors. T regulatory cells play a role in the expression of tolerance, and it is likely that these cells are stimulated initially by DCs (145, 311). T regulatory cells include those that secrete IL-10, TGF- $\beta$ , both IL-10 and TGF- $\beta$ , and those that suppress by cell-cell contact. The literature has given special names to T regulatory cells. For example, T regulatory 1 (Tr1) cells were described as cells that secreted IL-10 and inhibited T cell proliferation and cytokine secretion and IgE production in an antigen-specific manner, but did not affect either IgG<sub>1</sub> or IgG<sub>2a</sub> antibody responses (68). T regulatory cells in general are generated when responder T cells are stimulated repeatedly in the presence of high levels of IL-10 (128), a cytokine that can be secreted by DCs. A major role in oral tolerance for Peyer's patch IL-10-secreting DCs has been proposed (156).

TABLE 4. Factors that regulate DC IL-12 secretion

Stimulate IL-12	Suppress IL-12
CD40L (306)	PGE <sub>2</sub> (167, 355, 377)
IFN- $\gamma$ (366)	Chemokines MCP-1 to -4 (37)
Chemokine receptor ligands for CCR5 (7)	C5a (37)
Bacteria and bacterial products	Cytokines
LPS (349)	IL-10 (64)
CpG motifs (14, 26)	GM-CSF (342)
<i>Staphylococcus aureus</i> (138)	Corticosteroids (76)
Parasites	CD47 (thrombospondin receptor) ligation (83, 168, 183)
<i>Leishmania major</i> (104, 260)	Vitamin D <sub>3</sub> (259)
<i>L. donovani</i> (122)	Measles virus (110)
<i>Toxoplasma gondii</i> (252)	

Representative references are shown in parentheses. GM-CSF, granulocyte macrophage-colony stimulating factor.

### C. DC Role in B Cell Function

The focus of most studies with DCs is on their role in stimulating naive T cells, but DCs also play a role in stimulating B cells in both lymph node T cell areas and germinal centers (21, 93). A critical indirect role for DCs in B cell stimulation relates to their role in activating T cells to upregulate CD40L and secrete B cell helper factors. However, interdigitating DCs within the paracortical areas of the lymph node can interact directly with CD40-activated naive B cells to induce proliferation through an unidentified mechanism (93). Furthermore, in an IL-12-dependent mechanism, DCs contribute to B cell differentiation into IgM-secreting plasma cells (31, 94). Plasma cell differentiation is facilitated by DC secretion of the IL-6R  $\alpha$ -chain, gp80, which complexes to IL-6 and then binds to the IL-6R receptor on the responding B cell (94). DCs also stimulate CD40-ligand activated B cells to undergo isotype switching. DCs can capture and present unprocessed antigen to B cells and induce an IgG switch both in vitro and in vivo (383). Furthermore, human tonsillar interdigitating DCs induced CD40-ligated naive human B cells to secrete high levels of IgM antibody and to switch to both IgG and IgA isotypes. Interestingly, IL-13 was essential for Ig secretion, and DCs were the source of the IL-13 (160). The interaction of T cell area DCs with B cells may be facilitated by ELC secretion which attracts activated B cells as well as naive T cells to the DC microenvironment (247).

A unique follicular dendritic cell (FDC) population exists in the germinal centers of secondary lymphoid tissue and are important in B cell recall responses (348). FDCs are not bone marrow derived or related to the DCs being discussed in this review. However, FDCs are briefly discussed because they may be confused with the T cell-stimulating DCs. FDCs have abundant Fc and complement receptors that trap and retain immune complexes for long periods. The native antigen within the complexes binds the B cell receptor to reactivate memory B cells and, therefore, are likely important in maintaining serum antibody for long periods after exposures to infectious agents or effective vaccination. Furthermore, the complexes are fed to B cells as vesicular bodies called iccosomes, which are then processed and presented to follicular T cells to elicit their help. CD21 is a critical receptor on FDCs in stimulating B cells, because it binds immune complexes that have activated complement. Fc $\gamma$ RIIB is also important, because it reduces B cell coligation of the inhibitory Fc $\gamma$ RIIB on B cells with the B cell receptor (100, 267).

An additional DC subset identified within germinal centers of lymphoid organs in humans (and not related to FDCs) expresses CD4 and CD11c, but lacks the follicular center specific marker KiM4 (126). These cells are referred to as germinal center DCs (GCDCs) and ex vivo can

facilitate expansion of germinal center B cells already activated by CD40L and IL-2. B cell stimulation depended on IL-12 production by GCDCs (92). Furthermore, GCDCs were also capable of IL-10-independent isotype switching toward the Th2 antibody IgG1 (21). GCDCs secrete the CC chemokine DC-CK1 that has the ability to attract B cells and facilitate the interaction of the two cell types (199).

## VI. DENDRITIC CELLS AND INFECTIOUS DISEASE

### A. Microbial Subversion of DC Function

Microbes have cleverly learned to directly invade DCs in peripheral tissues and replicate intracellularly. By either a productive nonlytic infection and/or by killing DCs, the agent can then be spread locally, or infected DCs can carry the agent to draining lymph nodes. Furthermore, infectious agents can interfere with MHC class I and class II antigen processing and presentation pathways or activate T cells indiscriminately by presenting bacterial superantigens diverting an effective immune response (reviewed in Refs. 16, 21, 271, 331). Among organisms that have developed the ability to subvert DC function are the viruses human immunodeficiency virus I (HIV), Epstein-Barr virus (EBV), human choriomeningitis virus (HCMV), murine lymphocytic choriomeningitis virus (LCMV), human cytomegalovirus virus (HCMV), herpes simplex virus (HSV), and measles virus; the bacteria *M. tuberculosis*, *Yersinia enterocolitica*, *Salmonella* sp., and *Listeria monocytogenes*; and the parasites *Leishmania major*, *L. donovani*, and *Plasmodium falciparum* to name a few. For example, DC infection by both live and killed enteropathogenic *Y. enterocolitica* in vitro causes a transient suppression in their ability to stimulate both autologous CD4 and CD8 T cells (295). Selected studies will illustrate the principle that DCs are targets for immune regulation by infectious agents.

#### 1. HIV

HIV infection is one of the best-characterized infections with regard to exploitation of DCs. Persisting HIV infection leads to gradual destruction of T cells, the underlying cause of morbidity and mortality in acquired immunodeficiency syndrome (AIDS). DCs are early targets for infection by HIV at peripheral sites. In early infection, infectious HIV is macrophage-tropic and enters both DCs and monocyte/macrophages at mucosal sites by utilizing target cell surface CD4 and an essential coreceptor, the chemokine receptor CCR5 (16, 144, 294). Infected monocytes stimulated with GM-CSF and IL-4 develop into DCs, and subsequent exposure to LPS drives their maturation and migration to secondary lymphoid tissue where

they can readily infect CD4 T cells. In an in vitro coculture model of monocyte-derived, cultured DCs with autologous resting CD4 T cells, CCR5-tropic strains of HIV-1, but not CXCR4-tropic (or lymphotropic) strains, were transmitted to resting CD4 T cells, leading to productive viral replication, although DCs were susceptible to infection with either viral strain (75). Macrophages could be infected with both the CCR5 and CXCR4 tropic HIV strains but were unable to infect CD4 cells with either viral strain. Productive HIV infection appeared to result from formation of syncytia between infected DCs and T cells.

Several studies have reported a Th1/Th2 shift in the cytokine pattern from secretion of IL-2 and IFN- $\gamma$  toward IL-4 and IL-10 during progressive patient decline during an HIV-1 infection (reviewed in Ref. 62). Furthermore, the shift from CCR5 tropic to a CXCR4 tropic HIV-1 variant has been associated with disease progression (294). Data support the notion that the shift toward Th2 cytokines, particularly IL-4, downregulates CCR5 and upregulates CXCR4 expression on both DCs and CD4 T cells, allowing both to become infected with the more virulent lymphotropic strains of HIV (360, 371, 397). More recent studies show that IL-10 inhibits replication of CXCR4 tropic HIV-1 strains in macrophages, but significantly increases viral replication in DCs, thus acting in a similar fashion to IL-4 in facilitating development of more aggressive HIV disease (8). Others have studied mechanisms of enhanced HIV production during DC-T cell interactions. With the use of an HIV transgenic mouse model in which APCs served as the major source of inducible HIV expression, mechanisms by which integrated virus is activated intracellularly were investigated (61). When admixed with transgenic APCs, activated T lymphocytes provided a major contact-dependent stimulus for viral protein expression in vitro, which depended on CD40-CD40L interaction.

## 2. HCMV

HCMV is a DNA virus that persists in the human host following a primary infection, yet generally causes no pathology. The organism utilizes an array of different mechanisms that modulate the immune response to circumvent its clearance. These mechanisms include those that increase degradation of MHC class I and MHC class II molecules and that interfere with peptide trimming of antigens for presentation (206). Therefore, DCs are clearly targets for HCMV. Hematopoietic cells are important cellular sites for latent infections. Defined populations of myeloid lineage-committed progenitor cells were studied from HCMV seropositive humans to determine if they supported latency and under what conditions reactivation was achieved (132). Human fibroblasts cocultured with DCs and other myeloid cells supported latency and reactivation of the virus, although only very small

percentages of the myeloid cells were responsible (<0.01%). Cytokines that supported reactivation included IFN- $\gamma$ , TNF- $\alpha$ , IL-4, and GM-CSF, suggesting that immune-mediated inflammation could reactivate a HCMV infection. In another study, human monocyte-derived immature DCs were assessed as to whether they were susceptible to infection with HCMV (276). Immature DCs (80–90%) were susceptible to infection by HCMV strains that had been propagated in endothelial cell cultures. Furthermore, the DCs expressed viral immediate early, early, and late genes and supported a productive infection, which eventually led to destruction of the DCs in culture. Thus HCMV downregulates an effective immune response by destroying DCs. An example of how HCMV affects function in the absence of lytic infection of DCs is illustrated by a study examining the role of a HCMV product, the glycoprotein UL18. UL18 is a MHC class I homolog that binds to UL binding protein (ULBP), a glycosphosphatidylinositol-linked cell surface glycoprotein. UL18 interferes with ULBP binding to its receptor, the activating receptor, NKG2D/DAP10, on NK cells and therefore, UL18 blocks NK cell cytotoxicity (67). The consequence is that UL18 production by HCMV allows virally infected cells to evade attack by the innate immune system. UL18 also binds to the inhibitory leukocyte Ig-like receptor-1 (LIR-1), which belongs to a family of closely related immunoglobulin superfamily receptors. LIR-1 is expressed on B cells and myelomonocytic cells, including monocytes and DCs (65, 66). It is, therefore, possible that the UL18 molecule of CMV could interfere with physiological DC responses, perhaps interfering with controlled differentiation and cytokine release of DCs, subverting the negative regulatory function of LIR-1 and ultimately leading to HCMV persistence.

## 3. Measles virus

Mortality from measles virus infection in third world countries is caused mainly by secondary infections associated with a pronounced immunosuppression. DCs are an important target for the measles virus, which is able to subvert DCs to induce immunosuppression. LCs, CD34+ progenitor-derived DCs, and monocyte-derived DCs are all susceptible to infection with measles viruses. After infection, DCs can undergo maturation but fail to stimulate T cells (300). Measles virus-infected LCs or monocyte-derived DCs matured when exposed to TNF- $\alpha$  or LPS, but CD40L-dependent maturation of DCs was inhibited as demonstrated by decreased expression of CD40, CD80, CD86, and CD83. Moreover, the CD40L-induced cytokine pattern in measles virus-infected DCs demonstrated inhibition of IL-12. Finally, measles virus infection of DCs also prevented CD40L-dependent CD8 T cell proliferation. Another mechanism for measles virus immunosuppression involved induction of both DC and T cell

death by apoptosis (223). With the use of human monocyte-derived DCs, measles virus-infected DC-T cell cocultures resulted in DC apoptosis via Fas-FasL-mediated mechanisms. Dissection of the DC-T cell interactions revealed that CD40L expressed on activated T cells first enhanced measles virus replication in DCs, then FasL on activated T cells induced Fas-mediated apoptosis of DCs, which facilitated viral particle release. A second important observation was that apoptotic measles virus-infected DCs induced bystander maturation of uninfected DCs, a phenomenon that may be involved in the final initiation of a measles virus-specific response and was postulated to occur by the uptake of apoptotic, virally infected DCs (223).

#### 4. *Mycobacteria*

Pathogenic mycobacteria, including *M. tuberculosis*, cross mucosal barriers by endocytosis particularly in the oropharynx and nasopharynx, tonsils, and Peyer's patches (reviewed in Ref. 216). Bacilli that reach the basolateral surfaces of epithelial M cells are taken up by professional APCs. DCs and macrophages in these sites are permissive for mycobacterial replication, but cellular immunity develops that usually controls infection in the immunologically intact hosts. Nonetheless, phagocytes (and likely DCs) containing intracellular mycobacteria disseminate infection to other parts of the body.

#### 5. *Salmonella*

*Salmonella* sp. were shown to be capable of infecting and surviving within murine DCs (225). It has been suggested that DC infection by *Salmonella* might lead to dissemination of the organisms from the gut (331). A wild-type and three different attenuated strains of *Salmonella typhimurium* were all shown to be capable of infecting human monocyte-derived DCs (91). The wild-type and one attenuated strain persisted in the DCs longer than two attenuated strains, both of which were largely eliminated within 24 h. Most DCs survived infection by the attenuated strains, although apoptosis was observed in a fraction of the exposed cells. All strains induced DC maturation and stimulated IL-10, TNF- $\alpha$ , and IL-12. These studies are consistent with a role for *Salmonella*-infected DCs not only in stimulating immunity but also in dissemination and immunosuppression through the production of IL-10 and induction of limited APC apoptosis.

#### 6. *Listeria monocytogenes*

The sequence of cellular events leading to the dissemination of *L. monocytogenes* from the gut to draining mesenteric lymph nodes was studied by confocal microscopy of immunostained tissue sections in a rat ligated ileal loop system (261). *Listeria* were first detected in

DCs beneath the epithelia lining of Peyer's patches and then in draining mesenteric lymph nodes as early as 6 h after inoculation. Most bacteria (80–90%) were in deep paracortical regions, and all of the bacteria were present in cells compatible with DCs. The authors suggested that *Listeria* were transported by DCs from Peyer's patches to the deep paracortical regions of draining lymph nodes and from there transmitted to other cell populations. This study provided evidence that DCs may be involved in the early dissemination of this pathogen.

### B. Exploiting DCs for Vaccine Protocols

Current vaccines include toxoids, recombinant proteins and peptides emulsified in adjuvants, and completely inactivated or attenuated intact microbial agents. However, effective preventive strategies for many infectious agents have not been found, and no vaccines are yet available to enhance protective immunity against persisting infections. For example, infections with the intracellular pathogens *M. tuberculosis* and *L. donovani* are a worldwide problem. Despite effective therapy, in some cases disease progression may occur. An important corollary of the concept that microbes subvert DC function is that DCs and other APCs may be uniquely equipped to overcome persistence of microbes within the human host. Therefore, if DCs can be properly manipulated, they might become the most effective adjuvant to enhance the host's immune defenses and clear the infection (reviewed in Refs. 89, 271, 301, 328, 344). Among the many organisms that have been targeted for genetic vaccines in experimental systems are HIV, *Chlamydia* sp., *Borrelia burgdorferi*, LCMV, *Toxoplasma gondii*, *L. donovani*, and equine herpesvirus-1. DNA genetic vaccines may incorporate genes that encode microbial antigens and cytokines such as GM-CSF that enhance DC maturation or IL-12 that drives Th1 responses. Protocols have also been designed to directly inoculate DC vaccines as after DC exposure to microbial antigens so that they present antigens on both class I and class II MHC for generating both specific CD4 and CD8 T cells. This latter strategy has also been modified by transfecting antigen-pulsed DCs with cytokine constructs that facilitate their maturation to IL-12-secreting APCs.

In genetic vaccination, DNA encoding microbial genes are inoculated into the host. The genes are expressed within the host cells that take up the inoculated DNA. If the DNA enters a DC, the proteins are directly expressed within the cell and presented on MHC class I by the classical endogenous pathway. However, any transfected cell, including non-APCs, can secrete the microbial protein, thereby making antigen available for cross-presentation on DC MHC class I or for processing by the endocytic pathway for presentation on MHC class II. In

one study that supports this scenario, intradermal DNA injection of a CMV promoter-driven plasmid encoding model antigen induced sensitization against the encoded protein, and cutaneous DCs were shown to be involved in sensitization (36). DCs that migrated from the skin explant 3 days after the skin injection of the antigen construct contained mRNA encoding the antigen. The isolated DCs were capable of stimulating CD4 T cells and inducing cytolytic T cells, clearly establishing a role for skin DCs in genetic vaccination for both MHC class I and class II dependent immunity.

### 1. HCMV infection

HCMV infections are particularly serious in allogeneic transplant recipients, and therapy in this setting is often unsatisfactory. Recent studies have examined the feasibility of adoptive transfer of donor-derived virus-specific T cells generated in cultures with antigen-bearing autologous monocyte-derived DCs (256). APCs were pulsed with HCMV antigen and cocultured with autologous peripheral blood lymphocytes from HCMV-seropositive individuals. This process increased the numbers of cytolytic T cells, suggesting that this technique could potentially be useful for enhancing immunity in HCMV-seropositive transplant patients. One important caveat in using DCs as antigen-carrying cells for induction of immunity is that in general immature DCs process but fail to present, and mature DCs present but fail to process effectively through the class II endocytic pathway. Recombinant vaccinia viruses (VV) have been used to introduce antigens directly into immature DCs, but Bonini et al. (34) observed that this technique blocked DC maturation and, thus, these APCs were unable to generate an effective cytotoxic T cell response after infection with VV. On the other hand, recombinant VV-infected mature DCs induced a CD8 T cell response but were unable to stimulate CD4 cells, because the exogenous class II pathway was no longer intact. Using a clever strategy, the authors designed a gene containing the targeting sequence of a class II compartment-associated antigen [lysosomal-associated membrane protein 1 (LAMP1)], to guide a CMV antigen delivered on VV to the MHC class II compartment in mature DCs. These mature DCs expressed the HCMV antigen on both MHC class I and MHC class II, detected for at least 16 h, and were capable of stimulating both CD4 and CD8 T cells.

### 2. Tuberculosis

DC manipulation has been explored in an effort to enhance current immunization protocols against *M. tuberculosis* in murine infection models. DCs in the lung stimulate immunity to inhaled pathogens and so certainly are important in generating effective mycobacterial immunity. *Bacillus Calmette Guérin* (BCG) has been a stan-

dard component for immunizations against *M. tuberculosis* in many countries, although it is relatively ineffective. One study explored whether direct inoculation of BCG-infected DCs into the trachea would provide enhanced immunity in a murine model of infection compared with a subcutaneous BCG vaccination (78). As expected, BCG-infected DCs induced appropriate maturation of the DCs in vitro, including stimulating IL-12 secretion. After intratracheal instillation, the BCG-pulsed DCs induced a Th1 immune response in lung-draining lymph nodes. However, the response was no greater than that induced by subcutaneous vaccination. Nonetheless, clearly DCs bearing BCG antigens were immunogenic. Vaccination strategies that would optimize DC IL-12 production and their presenting the appropriate T cell epitopes should be successful. With the use of BCG-infected DCs, experiments were designed to activate DCs for enhanced IL-12 secretion via stimulating their CD40 with CD40L. CD40-stimulated BCG-infected DCs displayed increased capacity to release bioactive IL-12 and to activate IFN- $\gamma$ -producing T cells in vitro. However, although C57BL/6 mice immunized with BCG-infected, CD40-activated DCs demonstrated increased levels of type 1 cytokine production in vivo, the response did not increase lung resistance to intrapulmonary infection with virulent *M. tuberculosis* (79). One strategy might be to enhance CD8 T cell responses in addition to CD4 T cell responses. Thus, although CD4 T cells are essential for protective immunity against *M. tuberculosis* infection, CD8 T cells may also play a critical role, and strategies have been developed to design vaccines that contain epitopes recognized by both CD4 and CD8 T cells. In one study in which this issue was addressed, a VV construct expressing the mycobacterial antigen MPT64 was used to infect DCs and determine whether a CD8 T cells from BCG-infected mice could be activated by these DCs (102). The VV-MPT64-infected DCs were more effective than BCG-infected DCs in activating antigen-specific CD8 T cells to secrete IFN- $\gamma$  in vitro, suggesting that use of such peptide constructs might eventually be useful in genetic vaccines against *M. tuberculosis*.

### 3. Leishmaniasis

Using a murine model of visceral leishmaniasis, one successful approach to vaccine therapy was to couple the antigen presenting capacity of DCs with the paracrine delivery of IL-12 (5). DCs pulsed with soluble *L. donovani* antigens and inoculated into naive mice induced antigen-specific production of IFN- $\gamma$  and increased the percentage of activation markers on spleen lymphocytes. Antigen-pulsed DCs transfected with retroviral gene construct that encoded the biologically active murine IL-12 augmented the Th1 immune response. Finally, and most importantly,

in both vaccination and immunotherapy protocols, the doubly transfected DCs reduced the parasite burdens.

## VII. ROLE OF DENDRITIC CELLS IN IMMUNE-MEDIATED DISEASE

Because DCs are essential for initiating primary immune responses, they also play a central role in the initiation and perpetuation of immune-mediated disease. Hypersensitivity diseases include those that develop in response to both exogenous and endogenous (autoimmune) diseases. Asthma is an example of a hypersensitivity disease to exogenous antigens, i.e., allergens. Asthma is a Th2-mediated disease with both IgE antibody and type 2 cytokines playing effector roles in clinical exacerbations. In experimental models of asthma, DCs both initiate the primary response and play a role in the effector limb of the response (204, 248, 298). Many autoimmune diseases have been modeled in experimental animals; in many of these diseases, the mechanism of inflammatory damage is primarily related to the initiation and persistence of Th1 immune responses. Therefore, a role for DCs in this group of diseases is highly likely (215). Experimental autoimmune encephalomyelitis (EAE) has been elicited in both rats and mice and mimics the human neurologic disease multiple sclerosis. In EAE, strategies have successfully induced tolerance to both prevent and treat the disease. EAE is an example of an autoimmune disease in which a role for DCs has been established in causing the disease and in tolerance induction (201). Rather than review the literature for a role of DCs in a large group of hypersensitivity and autoimmune diseases, we focus on asthma and EAE to illustrate the principles of how DCs participate in these diseases.

### A. Asthma: a Th2-Mediated Inflammatory Lung Disease

#### 1. Lung DCs

In the lung, DCs reside within and beneath airway epithelium, in alveolar septae, in the connective tissue surrounding pulmonary veins and airway vessels, and within the lung capillaries of the lung parenchyma (reviewed in Ref. 202). DCs in the airway epithelium express an immature phenotype and exhibit a rapid turnover (142, 233). DCs residing within alveolar septae and in connective tissue surrounding vessels have a more mature phenotype than airway DCs (121). DCs residing within the lung vascular compartment represent circulating precursor DCs (336). Our studies in mice indicate that most lung DCs are CD8 $\alpha$ <sup>-</sup> and express CD11c and CD11b (228). One role of lung DCs is to provide protection against infectious agents by initiating type 1 immune response.

An equally important role is to generate tolerance to inhaled allergens in normal noninflamed lungs. In this regard, immature DCs continuously leave the peripheral blood and assume a surveillance position in lung tissue, avidly sampling the antigenic environment. In the steady state, lung DCs likely remain relatively immature and constitutively migrate in low numbers into regional lymph nodes where they induce either anergy, deletion of T cells, or a weak Th2-like response to air-borne antigens that eventually is downregulated (334). Most humans and experimental animals fail to respond to inhaled allergens with allergic pulmonary inflammation because they either develop tolerance or simply fail to respond immunologically. Indeed, the failure of immature DCs to become mature DCs in response to inhaled allergens may be the primary reason most humans are tolerant to inhaled non-microbial antigens (334). Active suppression of immature DC maturation by alveolar macrophages may explain why airway and intraepithelial DCs remain immature during their steady-state migration to lung-associated lymph nodes (LALNs) (141, 203). Furthermore, autocrine production of IL-10 by immature DCs can inhibit surface expression of MHC class II and exert a generalized inhibitory effect on T cell proliferation (238, 334). On exposure to inhaled allergens, the antigen may simply be insufficient in providing a danger signal to overcome suppression by alveolar macrophages and IL-10. However, if a danger signal is present at the tissue site, DCs mature and migrate in greater numbers to the draining lymph nodes to stimulate CD4 T cell clonal expansion and differentiation. In most infections, polarization of maturing DCs into DCs that can produce IL-12 occurs. DCs in draining lymph nodes may also be stimulated to produce IL-12 in lymph nodes during an ongoing T cell response when CD40 on DCs is cross-linked by CD40L on responder T cells (137, 168, 306), if factors that suppress IL-12 secretion are absent within the lymph node.

#### 2. Lung DCs in priming and eliciting pulmonary Th2 response

The role of lung DCs in providing protection against infectious agents through the strong promotion of type 1 immunity may be a key to establishing a microenvironment in the lung that suppresses the development of allergen-specific Th2 responses. Allergen exposure during an ongoing Th1 response in the lung could potentially drive an allergen-specific Th1 response or at least prevent development of an allergen-specific Th2 response. This might occur if the Th1 response drove allergen-presenting immature DCs to mature into IL-12-secreting DCs. In a study of children in Japan, the maintenance of a strong purified protein derivative (PPD) immune response was associated with protection from asthma, which suggested that the local or systemic host response to the tubercle

bacillus deviated responses to allergens away from Th2 responses (304). Furthermore, BCG given to mice before systemic antigen priming and aerosol challenge protected against the development of allergic pulmonary inflammation (95, 136). Alternatively, inflammation provoked by environmental toxicants or infections may result in the production of PGE<sub>2</sub>, IL-10, or TGF- $\beta$  by lung cells in the microenvironment of immature DCs and cause them to mature into DCs incapable of secreting IL-12. Childhood respiratory viral infections, particularly respiratory syncytial virus (RSV) infections, increase the risk of developing asthma (177, 308). Speculation is that during RSV infections, lung DCs may be induced to mature, yet be incapable of secreting IL-12. Thus allergen-carrying DCs that reach the lung-draining lymph nodes are more likely to initiate a primary allergen-specific Th2 response (Fig. 5). All children who experience RSV bronchiolitis do not develop asthma. Therefore, implicit in this hypothetical sequence is that genetic factors influence the host response to RSV and, thus, whether the lung microenvironment can mediate DC differentiation that drives a strong Th2 response to allergen. In one recent study, antigen-pulsed splenic DCs were inoculated intratracheally into naive mice and primed mice for airway hyperresponsiveness, lung eosinophilia and inflammation, and mucous cell metaplasia, suggesting that the normal lung environment is conducive to developing Th2 rather than Th1 responses (338).

DCs also likely play a role in elicitation of pulmonary allergic inflammation. Lambrecht et al. (185) demonstrated a critical role for lung DCs in the effector limb of inflammation in a murine allergic inflammation model. The investigators used a thymidine kinase-transgenic mouse in which DCs were exquisitely sensitive to destruction when mice were treated with the antiviral drug ganciclovir. The mice were immunized with OVA in a standard Th2-producing protocol in which the antigen is inoculated intraperitoneally in alum. Once memory Th2 cells were generated, the depletion of lung DCs before a secondary exposure to OVA via the respiratory tract markedly reduced the expected Th2-mediated inflammatory response.

### 3. Role for lung DCs in the persistence of asthma

The total number of lung DCs is increased in bronchial tissue of asthmatics (27, 237), although it is not known whether these lung DCs are phenotypically and functionally different from those present in the bronchi and bronchioles of nonasthmatic individuals. In these studies, treatment with glucocorticoids significantly reduced the number of DCs to levels seen in nonasthmatic people. These findings suggest that the increased

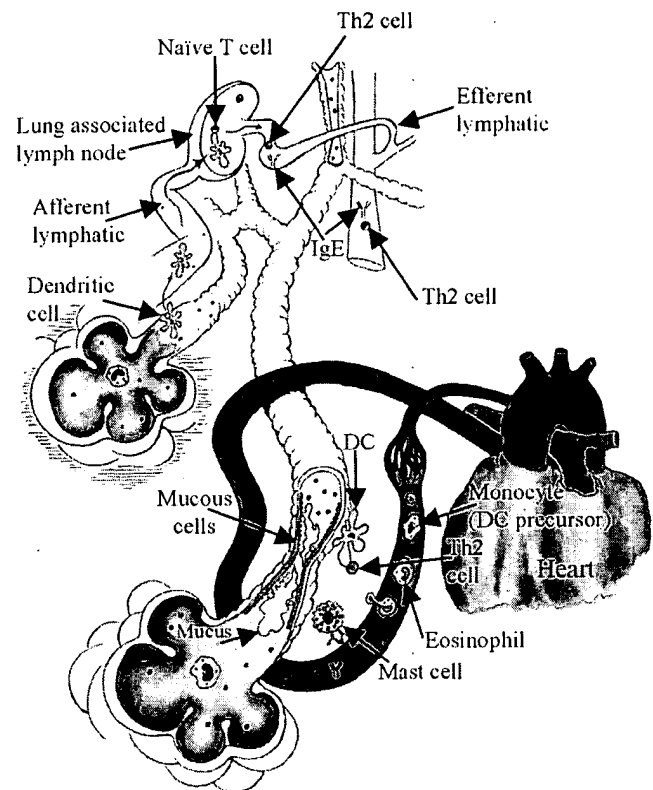


FIG. 5. Role of DC in asthma. In a naive host (upper bronchus) during the afferent limb of the immune response, allergen is inhaled, taken up by DCs, and carried to the LALNs in the context of a Th2-enhancing microenvironment. During the central processing phase of the immune response in LALNs, mature DCs are unable to make IL-12 and present allergen to naive, antigen-specific T cells. This interaction results in clonal T cell expansion and differentiation to allergen-specific Th2 T cells. Th2 cells drive B cells to proliferate and differentiate into IgE-secreting cells. Both IgE and Th2 cells leave LALNs via efferent lymphatics and reach the lung (lower bronchus), where an allergic response will occur on reexposure to the immunizing allergen. In the effector limb of the pulmonary asthmatic response, IgE and antigen cross-link Fc $\epsilon$ R1s on mast cells, signaling an acute phase response. Resident DCs also present allergen-specific T cell epitopes bound to class II to resident Th2 cells that are stimulated to participate in late phase asthmatic responses by secreting Th2 cytokines. Repeated allergen exposures lead to chronic inflammation, possibly enhanced by the recruitment of monocytes and other precursor DCs that, in the setting of ongoing Th2-mediated inflammation, become effective resident DCs.

numbers of DCs in asthmatic airways could be an important factor in the persistence of chronic T cell-mediated allergic inflammation. Once allergen-specific Th2 memory cells are drawn into the lung, repeated allergen presentation by lung DCs may drive the persistent stimulation of allergen-specific memory Th2 cells (Fig. 5). This repeated Th2 cell stimulation would be expected to exacerbate acute asthma episodes and to perpetuate the state of chronic inflammation that contributes to the remodeling and airway hyperreactivity that characterize chronic asthma.

## B. EAE: a Th1-Mediated Disease

### 1. DCs in initiating immune-mediated central nervous system inflammation

EAE is an experimental demyelinating disease induced in rodents by immunization against myelin-specific proteins. EAE is mediated by antigen-specific T cells. This knowledge was established by adoptive transfer experiments and the demonstration that B cells and antibody do not play a role (139, 253). The T cells in EAE secrete IFN- $\gamma$ , and recruited macrophages secrete TNF- $\alpha$ ; both cytokines are important in mediating central nervous system (CNS) inflammation. During the induction of the immune response to myelin proteins, DCs undoubtedly induce naive T cell proliferation and polarization. During expression of EAE in the CNS after priming, T cells must also recognize antigen presented by CNS APCs. CNS APCs must upregulate MHC class II, because in the normal state they only minimally express it. The regulation of expression of the class II transactivator (CIITA) required for activation of MHC class II genes was, therefore, studied in EAE (340). EAE was induced in C57BL/6 mice by immunization with a myelin oligodendrocyte glycoprotein peptide (MOG). The DC-specific form of CIITA, but not the B cell-specific form, was detected in the CNS of mice with acute EAE. DCs were identified by immunostaining for CD11c in perivascular and meningeal cell infiltrates in the CNS, and the time course of their appearance was compatible for their role in the evolution of the disease. In another study of EAE in mice, infiltrating CD11b+ macrophages and resident microglia were shown to present antigens to T cells to stimulate IFN- $\gamma$  production, and CD11b- cells were inefficient APCs (164). Interestingly, CD11c+ cells, putatively DCs, were present in the both the CD11b+ and CD11b- populations, but in this study comprised only 5 and 4% of the APC populations, respectively, thus questioning an important role for DCs in the effector limb of EAE. In another EAE study, more convincing evidence that DCs were recruited to the CNS in the effector phase of EAE was found (299). After immunization of mice with a relevant peptide, cells with a dendritic shape and expressing the DC markers DEC205 and CD11c appeared in the spinal cord. During acute, chronic, and relapsing stages of the disease, DCs expressing high-density MHC class II and costimulatory molecules accumulated within the inflammatory cell infiltrates in the CNS. MIP-3 $\alpha$ , a chemokine known to attract DCs and lymphocytes, and the MIP-3 $\alpha$  receptor, CCR6, were both upregulated in the CNS during the disease. In a fourth murine EAE model study, among CD11b+ brain cells, significant numbers of cells that exhibited the DC marker CD11c were found, constituting up to 30% of the total CD11b+ brain cell population (103). The CD11c+ cells displayed the surface phenotype of myeloid DCs,

resided at perivascular and parenchymal inflammatory sites, and lacked prominent phagocytic organelles. The brain DCs secreted IL-12p70 and were potent stimulators of naive and allogeneic T cell proliferation. Both DCs and CD11c-negative CD11b+ microglia/macrophages from inflamed brain primed naive T cells from DO11.10 TCR transgenic mice for production of Th1 cytokines, indicating that in EAE, DCs do play a role, but not an exclusive one, in driving T cell-dependent inflammation.

### 2. DCs in immunotherapy of EAE

DC-based immunotherapy has been proposed as an adjunct to conventional therapy in autoimmune diseases (reviewed in Ref. 200). In a Lewis rat model of EAE, bone marrow-derived DCs pulsed with encephalitogenic myelin basic protein peptide and injected subcutaneously before immunization prevented the development of EAE (146). Tolerance was associated with augmented inducible nitric oxide synthase (iNOS) expression and NO production and an increase in apoptotic cells. To determine the distinguishing properties of tolerogenic DCs, the authors separated the bone marrow-derived cells into adherent and nonadherent populations (386). They found that the adherent population was able to mediate tolerance, whereas the nonadherent population was not. DC-mediated tolerance to EAE following inoculation of the adherent DCs was associated with an increase in NO production, apoptotic cells, and TGF- $\beta$  expressing cells in T cell areas of lymph nodes.

## VIII. ROLE OF DENDRITIC CELLS IN TRANSPLANTATION WITH THERAPEUTIC IMPLICATIONS

DCs play an essential role in initiating the host-versus-graft immune response and ultimately determine organ graft failure or sustained engraftment (reviewed in Refs. 99, 240, 350, 351). DCs also initiate graft-versus-host disease (GVHD) by presenting incompatible recipient antigens to donor T cells after allogeneic bone marrow transplantation (114, 243). Current methods used for the prevention of GVHD include in vitro T cell depletion of the graft and in vivo prophylactic immunosuppression (18, 390). This section emphasizes the immunobiology of DCs in host-versus-graft recognition rather than GVHD.

In commonly transplanted organs such as liver, heart, kidney, and skin, donor DCs are present as immature cells and persist indefinitely in successful liver transplants (17, 190, 321, 322, 379). Recipient DCs are also found infiltrating allografts as part of the inflammatory reaction that takes place after the transplantation surgery. Both graft-derived "passenger" DCs and graft-infiltrating recipient DCs can exert control over the outcome of the transplant, leading to acute rejection or acceptance of the

allograft (99, 123, 240, 351). Acute rejection of allografts occurs because recipient T cells mount a strong specific immune response against donor allogeneic antigens, and long-term acceptance of allografts occurs because recipient T cells become tolerant to donor allogeneic antigens (350). Donor DCs leave the engrafted organ and migrate to draining lymphoid organs where they present allogeneic MHC molecules to recipient T cells (190, 240, 321, 322). The frequency of allospecific responder T cells to donor peptide complexed with donor MHC is high, leading to clonal T cell expansion. Recipient DCs that enter the graft strongly influence the outcome of the transplant by internalizing, processing, and presenting donor antigens to host T cells. In doing so, recipient DCs present a restricted repertoire of immunodominant allogeneic peptides bound to self-MHC molecules to a low frequency (similar to the frequency of T cells responding to nominal antigen peptides) of recipient T cells. A source of donor antigen is thought to be apoptotic and/or necrotic donor cells (111). DCs process donor antigens and present allogeneic peptides bound to both MHC class I (cross-priming) and class II molecules for presentation to CD8 and CD4 recipient T cells, respectively. Immature DCs employ a unique translocator within the endosomal membrane that allows endocytosed antigens access to the cytosol and to the conventional MHC class I antigen-presenting pathway (277).

The role donor and recipient DCs play in allograft rejection seems to depend on the type of organ or tissue being transplanted and the experimental animal model (123). For example, both donor and recipient DCs are involved in acute rejection of skin allografts in rat and mouse models (15, 275, 361). In contrast, in rodent models, recipient DCs seem to play key roles in the rejection of cardiac allografts, while donor DCs appear to be required for the induction of tolerance (162, 173, 220). Pretreatment of MHC-disparate recipients with immature monocyte-derived donor DCs propagated in the presence of GM-CSF resulted in prolonged cardiac allograft survival in a rat model (109). Pretreatment of recipients with mature DCs before transplantation failed to prolong graft survival. The eventual rejection of the allograft in rats receiving immature donor DCs was thought to be due to the maturation of donor DCs *in vivo*.

Early inflammatory events in organ and tissue allografts also shape their future. The maturation state and ability to secrete IL-12 by graft-derived and recipient infiltrating DCs may be determined by the local microenvironment and determines the way responding T cells react to the allogeneic antigen. Proinflammatory cytokines released locally in the graft could increase expression of MHC molecules and costimulatory molecules on graft-derived and recipient infiltrating DCs. Death also presents a danger signal that programs DCs to mature and secrete IL-12. Therefore, both donor and recipient DCs are pro-

grammed to drive the differentiation of Th0 cells to Th1 cells and to initiate acute rejection.

However, acute rejection is not always the outcome. Successful liver transplantation induces donor-specific tolerance across major MHC barriers without prophylactic immunosuppression (350). The microenvironment in the liver dampens the proinflammatory effects of cytokines released after transplantation. The liver has several unique biological features that influence the properties of local DCs. First, the liver is a hematopoietic organ capable of giving rise to all leukocyte lineages, and after allogeneic liver transplantation, donor hematopoietic cells can be propagated *in vitro* from the bone marrow of recipients (213). Second, parenchymal and stromal cells of the liver are capable of producing IL-10, TGF- $\beta$ , and GM-CSF that are known to modify the growth and function of DCs (168). As discussed, IL-10 reduces expression of MHC class II and costimulatory molecules and blocks IL-12 synthesis by DCs (44, 168, 171). TGF- $\beta$  is a potent immunosuppressive cytokine that can block DC maturation (168, 384). Together, these two cytokines inhibit the maturation and IL-12 production of DCs trafficking to recipient draining lymph nodes and instead regulate DCs to drive the differentiation of Th0 cells into Th2 cells and/or regulatory T cells that reinforce the state of tolerance (99). Under the influence of GM-CSF, graft-derived DCs migrate rapidly from the graft to T cell-dependent areas of recipient draining lymph nodes to induce allogeneic-specific unresponsiveness in recipient murine T cells (211, 352). A component of immune unresponsiveness seems to be activation-induced cell death (AICD) among alloreactive T cells, critical for the establishment of tolerance to allografts (196, 266, 374). T cells undergoing AICD secrete large quantities of IL-10 before their death, which may affect DC function by inhibiting the expression of MHC class II and costimulatory molecules on DCs (113). T cells surviving AICD are proposed to be regulatory T cells that secrete an unusual combination of cytokines, including IL-10 and IFN- $\gamma$  (128, 374). DCs are thought to be important in AICD, because they express members of the TNF family known to induce apoptosis, including FasL and TRAIL (101, 212). Experimental evidence suggests that Fas-FasL interactions are also needed to secure a state of tolerance by donor bone marrow in bone marrow transplantation (118).

A major goal in transplantation research is to understand and exploit tolerogenic DCs. Several approaches to harnessing the tolerogenic potential of DCs involve administration of immature donor DCs in conjunction with molecules that block costimulation, *i.e.*, CTLA4Ig, anti-CD40L, *ex vivo* manipulation of donor-derived DCs to generate costimulatory deficient immature DCs (precursor DCs propagated in the presence of TGF $\beta$ ), and genetic engineering of donor-derived DCs to express immunoregulatory cytokines, *i.e.*, TGF- $\beta$ , IL-10, AICD-inducing

ligands such as FasL, or molecules that interfere with costimulatory pathways, i.e., CTLA4Ig, CD40Ig, CTLA4L (172, 194, 207–210, 212, 230, 236, 345, 392). In addition, bone marrow-derived DCs with reduced allostimulatory ability have been generated *in vitro* using cyclosporin A to block the nuclear translocation of the transcription activator NF $\kappa$ B, which controls the transcription of genes encoding MHC and costimulatory molecules (193). Together, these studies suggest the feasibility of reducing the allostimulatory capacity of donor DCs and, in some cases, enhancing their ability to drive Th2 allogeneic responses. However, as promising as the therapeutic exploitation of DCs seems to be, the success of these experimental DCs in establishing long-term allograft survival, indicative of strong tolerance, must still be established.

## IX. ROLE OF DENDRITIC CELLS IN CANCER WITH THERAPEUTIC IMPLICATIONS

The interest in the role of DCs in cancer immunosurveillance and immunotherapy is extraordinary, with over 1,500 articles listed in a search for articles on DCs and cancer, including 300 reviews, since 1992. Neoplastic disease represents the escape of cellular growth from normal host regulatory mechanisms. Successful cancers are able to circumvent the immune system by several mechanisms, for example, by inducing apoptosis in DCs so that they are unavailable to generate a productive immune response (96, 97, 354). An important property of tumor cells is that they typically do not release danger signals so that even if immature DCs take up and process shed tumor antigens or apoptotic tumor cells, they may fail to mature. Therefore, these DCs would be expected to induce tolerance rather than productive immunity (312). Other tumor escape mechanisms from the immune system have been identified and are described in recent reviews in which the possibilities of using genetically engineered DCs to enhance host immunity is discussed (43, 72, 106). Cancer immunotherapeutic strategies include genetic vaccination with DNA encoding tumor specific genes and activating host DCs *in situ* to enhance tumor resistance by intratumoral or systemic inoculation of DC-mobilizing cytokines such as GM-CSF or Flt3L. One of the more interesting approaches, and one being widely examined, is to directly load DCs with tumor antigens *in vitro* and then inoculate the DCs into the tumor-bearing host. Antigen loading has been achieved using tumor peptides or by transfecting with DNA or RNA encoding the antigens. Other antigen-loading strategies use apoptotic tumor cells or antigen-pulsed DC-derived exosomes, which are vesicles that contain high concentrations of MHC, peptide, and costimulatory molecules. The important outcome with all of these techniques is that tumor antigens are processed by both the endocytic and

proteosomal DC pathways and are capable of stimulating both CD4 and CD8 T cells. Other genes have been transfected into DCs to enhance their immunogenicity by stimulating expression of costimulatory molecules, enhancing cytokine secretion, and inducing apoptosis resisting ligands.

Clinical trials using DC-based tumor vaccines have generally demonstrated relatively few side effects, with mild fever being most common (217). One challenge of DC-based tumor vaccines is the difficulty of measuring that a relevant immune response has been induced. Thus one would wish to identify the frequency of tumor-specific cytotoxic T cells and tumor-specific CD4 and CD8 T cells capable of producing inflammatory cytokines (reviewed in Ref. 296). Another obstacle in the use of tumor vaccines, whether DC based or not, is the risk of causing autoimmunity against nontumor host cells (214). This side effect was described in 1 of 14 volunteers in a melanoma vaccine trial in which DCs pulsed with melanoma antigens caused vitiligo (217).

Despite the difficulties, efforts continue to perfect DC-based antitumor vaccines. Phase I clinical trials have established the feasibility of this approach against a number of human tumors, including renal cell carcinoma, melanoma, prostate carcinoma, cervical carcinoma, breast carcinoma, ovarian carcinoma, multiple myeloma, and intracranial glial tumors (2, 12, 41, 105, 158, 197, 246, 353, 356, 358). The success in causing remission in patients on these trials has been modest, but the lack of success in treating advanced cancer by other modalities is also an acknowledged medical challenge. Therefore, it is likely that over the next five years or so, for at least some tumors, DC vaccines will find a role.

## IX. SUMMARY AND FUTURE DIRECTIONS

Since the first description of a novel splenic cell by Ralph Steinmen in 1973, the understanding of DCs and their pivotal role in immune regulation and disease has grown exponentially. They are truly nature's most potent adjuvant for initiating immunity. Scientists had not anticipated that these cells would become useful for generating productive immunity to control a multiplicity of infections and cancer and to induce tolerance for treatment of allergic and autoimmune diseases and to prevent transplant rejection. At least for the foreseeable future, biomedical researchers and vaccine developers will undoubtedly continue to seek ways to exploit their biology for these medical indications.

We acknowledge our debt to Melissa Roy for her dedication and expert help in the preparation of this manuscript, to Andrea Draper for creating Figure 5, and to Michael Grady for creating Figure 4 and helping to finalize other figures. We also thank our many colleagues, particularly Drs. Julie Wilder, Rick

Lyons, Mark Eichinger, Jan Oliver, Bridget Wilson, Chris Kepley, and Mark Schuyler for fruitful discussions over the last two years as we have developed a growing appreciation for a role for dendritic cells in pulmonary immune regulation and in asthma.

We gratefully acknowledge funding for our asthma research through National Heart, Lung, and Blood Institute-funded Specialized Center for Research in Asthma Grant P50-HL-56384, an American Lung Association Asthma Research Center grant, and institutional support from the New Mexico tobacco tax research allocation to the University of New Mexico.

Address for reprint requests and other correspondence: M. F. Lipscomb, Dept. of Pathology, BMSB, Room 337, 915 Camino de Salud NE, Albuquerque, NM 87131-5301 (E-mail: mlipscomb@salud.unm.edu).

## REFERENCES

1. ABBAS AK, LICHTMAN AH, AND POBER JS. *Lymphocyte Maturation and Expression of Antigen Receptor Genes*. Philadelphia, PA: Saunders, 2000.
2. ADAMS M, BORYSIEWICZ L, FLANDER A, MAN S, JASANI B, NAVABI H, LIPETZ C, EVANS AS, AND MASON M. Clinical studies of human papilloma vaccines in pre-invasive and invasive cancer. *Vaccine* 19: 2549–2556, 2001.
3. ADEMA GJ, HARTIGERS F, VERSTRATEN R, DE VRIES E, MARLAND G, MENON S, FOSTER J, XU Y, NOOYEN P, MCCLANAHAN T, BACON KB, AND FIGDOR CG. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 387: 713–717, 1997.
4. ADEREM A AND ULEVITCH RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 406: 782–787, 2000.
5. AHUJA SS, REDDICK RL, SATO N, MONTALBO E, KOSTECKI V, ZHAO W, DOLAN MJ, MELBY PC, AND AHUJA SK. Dendritic cell (DC)-based anti-infective strategies: DCs engineered to secrete IL-12 are a potent vaccine in a murine model of an intracellular infection. *J Immunol* 163: 3890–3897, 1999.
6. AL-ALWAN MM, ROWDEN G, LEE TD, AND WEST KA. The dendritic cell cytoskeleton is critical for the formation of the immunological synapse. *J Immunol* 166: 1452–1456, 2001.
7. ALBERTI J, REIS E SOUSA C, SCHITO M, HIENY S, WELLS T, HUFFNAGLE GB, AND SHER A. CCR5 provides a signal for microbial induced production of IL-12 by CD8 alpha+ dendritic cells. *Nat Immunol* 1: 83–87, 2000.
8. ANCUTA P, BAKRI Y, CHOMONT N, HOCINI H, GABUZDA D, AND HAEFFNER-CAVAILLON N. Opposite effects of IL-10 on the ability of dendritic cells and macrophages to replicate primary CXCR4-dependent HIV-1 strains. *J Immunol* 166: 4244–4253, 2001.
9. ANDERSON DC, MILLER LJ, SCHMALSTIEG FC, ROTHLEIN R, AND SPRINGER TA. Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessments employing subunit-specific monoclonal antibodies. *J Immunol* 137: 15–27, 1986.
10. ANDERSON KV. Toll signaling pathways in the innate immune response. *Curr Opin Immunol* 12: 13–19, 2000.
11. ANJUERE F, MARTIN P, FERRERO I, FRAGA ML, DEL HOYO GM, WRIGHT N, AND ARDAVIN C. Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood* 93: 590–598, 1999.
12. APOSTOLOPOULOS V, PIETERSZ GA, AND MCKENZIE IF. MUC1 and breast cancer. *Curr Opin Mol Ther* 1: 98–103, 1999.
13. ARDAVIN C. Thymic dendritic cells. *Immunol Today* 18: 350–361, 1997.
14. ASKEW D, CHU RS, KRIEG AM, AND HARDING CV. CpG DNA induces maturation of dendritic cells with distinct effects on nascent and recycling MHC-II antigen-processing mechanisms. *J Immunol* 165: 6889–6895, 2000.
15. AUCHINCLOSS H JR, LEE R, SHEA S, MARKOWITZ JS, GRUSBY MJ, AND GLIMCHER LH. The role of "indirect" recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. *Proc Natl Acad Sci USA* 90: 3373–3377, 1993.
16. AUSTYN JM. Antigen-presenting cells. Experimental and clinical studies of dendritic cells. *Am J Respir Crit Care Med* 162: S146–S150, 2000.
17. AUSTYN JM, HANKINS DF, LARSEN CP, MORRIS PJ, RAO AS, AND ROAKE JA. Isolation and characterization of dendritic cells from mouse heart and kidney. *J Immunol* 152: 2401–2410, 1994.
18. AVERSA F, TABILIO A, VELARDI A, CUNNINGHAM I, TERENZI A, FALZETTI F, RUGGERI L, BARBABIETOLA G, ARISTEI C, LATINI P, REISNER Y, AND MARTELLI MF. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med* 339: 1186–1193, 1998.
19. BACHMANN MF, MCKALL-FAIENZA K, SCHMITS R, BOUCHARD D, BEACH J, SPEISER DE, MAK TW, AND OHASHI PS. Distinct roles for LFA-1 and CD28 during activation of naive T cells: adhesion versus costimulation. *Immunity* 7: 549–557, 1997.
20. BAGGIOLINI M. Chemokines and leukocyte traffic. *Nature* 392: 565–568, 1998.
21. BANCHEREAU J, BRIERE F, CAUX C, DAVOUST J, LEBECQUE S, LIU YJ, PULENDRAN B, AND PALUCKA K. Immunobiology of dendritic cells. *Annu Rev Immunol* 18: 767–811, 2000.
22. BANCHEREAU J AND STEINMAN RM. Dendritic cells and the control of immunity. *Nature* 392: 245–252, 1998.
23. BASU S, BINDER RJ, RAMALINGAM T, AND SRIVASTAVA PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* 14: 303–313, 2001.
24. BASU S, BINDER RJ, SUTO R, ANDERSON KM, AND SRIVASTAVA PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int Immunol* 12: 1539–1546, 2000.
25. BASU S AND SRIVASTAVA PK. Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum, elicits tumor- and peptide-specific immunity. *J Exp Med* 189: 797–802, 1999.
26. BAUER M, REDECKE V, ELLWART JW, SCHERER B, KREMER JP, WAGNER H, AND LIPFORD GB. Bacterial cpG-dna triggers activation and maturation of human cd11c(-), cd123(+) dendritic cells. *J Immunol* 166: 5000–5007, 2001.
27. BELLINI A, VITTORI E, MARINI M, ACKERMAN V, AND MATTOLI S. Intra-epithelial dendritic cells and selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest* 103: 997–1005 1993.
28. BENDELAC A, LANTZ O, QUIMBY ME, YEWDELL JW, BENNINK JR, AND BRUTKIEWICZ RR. CD1 recognition by mouse NK1+ T lymphocytes. *Science* 268: 863–865, 1995.
29. BINDER RJ, ANDERSON KM, BASU S, AND SRIVASTAVA PK. Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+ cells in vivo. *J Immunol* 165: 6029–6035, 2000.
30. BINDER RJ, HAN DK, AND SRIVASTAVA PK. CD91: a receptor for heat shock protein gp96. *Nat Immunol* 1: 151–155, 2000.
31. BJORCK P, FLORES-ROMO L, AND LIU YJ. Human interdigitating dendritic cells directly stimulate CD40-activated naive B cells. *Eur J Immunol* 27: 1266–1274, 1997.
32. BLAUVELT A, ASADA H, KLAUS-KOVTUN V, ALTMAN DJ, LUCEY DR, AND KATZ SI. Interleukin-15 mRNA is expressed by human keratinocytes Langerhans cells, and blood-derived dendritic cells and is down-regulated by ultraviolet B radiation. *J Invest Dermatol* 106: 1047–1052, 1996.
33. BLUM B, HO S, ANTONENKO S, AND LIU YJ. Generation of interferon alpha-producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. *J Exp Med* 192: 1785–1796, 2000.
34. BONINI C, LEE SP, RIDDELL SR, AND GREENBERG PD. Targeting antigen in mature dendritic cells for simultaneous stimulation of CD4(+) and CD8(+) T cells. *J Immunol* 166: 5250–5257, 2001.
35. BORKOWSKI TA, LETTERIO JJ, FARR AG, AND UDEY MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. *J Exp Med* 184: 2417–2422, 1996.
36. BOULOC A, WALKER P, GRIVEL JC, VOGEL JC, AND KATZ SI. Immunization through dermal delivery of protein-encoding DNA: a role for migratory dendritic cells. *Eur J Immunol* 29: 446–454, 1999.
37. BRAUN MC, LAHEY E, AND KELSALL BL. Selective suppression of IL-12 production by chemoattractants. *J Immunol* 164: 3009–3017, 2000.

38. BRENNER M AND PORCELLI S. Antigen presentation: a balanced diet. *Science* 277: 332, 1997.
39. BROCKER T. The role of dendritic cells in T cell selection and survival. *J Leukoc Biol* 66: 331–335, 1999.
40. BROCKER T, RIEDINGER M, AND KARJALAINEN K. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *J Exp Med* 185: 541–550, 1997.
41. BROSSART P, WIRTHS S, STUHLER G, REICHARDT VL, KANZ L, AND BRUGGER W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. *Blood* 96: 3102–3108, 2000.
42. BRUNO L, RES P, DESSING M, CELLA M, AND SPITS H. Identification of a committed T cell precursor population in adult human peripheral blood. *J Exp Med* 185: 875–884, 1997.
43. BUBENIK J. Genetically engineered dendritic cell-based cancer vaccines (review). *Int J Oncol* 18: 475–478, 2001.
44. BUELENS C, WILLEMS F, DELVAUX A, PIERARD G, DELVILLE JP, VELU T, AND GOLDMAN M. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. *Eur J Immunol* 25: 2668–2672, 1995.
45. BURGSTAHLER R, KEMPKE B, STEUBE K, AND LIPP M. Expression of the chemokine receptor BLR2/EB1 is specifically transactivated by Epstein-Barr virus nuclear antigen 2. *Biochem Biophys Res Commun* 215: 737–743, 1995.
46. CASTELLINO F AND GERMAIN RN. Extensive trafficking of MHC class II-invariant chain complexes in the endocytic pathway and appearance of peptide-loaded class II in multiple compartments. *Immunity* 2: 73–88, 1995.
47. CAUX C, DEZUTTER-DAMBUYANT C, SCHMITT D, AND BANCHEREAU J. GM-CSF and TNF- $\alpha$  cooperate in the generation of dendritic Langerhans cells. *Nature* 360: 258–261, 1992.
48. CAUX C, MASSACRIER C, DUBOIS B, VALLADEAU J, DEZUTTER-DAMBUYANT C, DURAND I, SCHMITT D, AND SAELAND S. Respective involvement of TGF- $\beta$  and IL-4 in the development of Langerhans cells and non-Langerhans dendritic cells from CD34<sup>+</sup> progenitors. *J Leukoc Biol* 66: 781–791, 1999.
49. CAUX C, MASSACRIER C, VANBERVLIET B, DUBOIS B, DURAND I, CELLA M, LANZAVECCHIA A, AND BANCHEREAU J. CD34<sup>+</sup> hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor  $\alpha$ . II. Functional analysis. *Blood* 90: 1458–1470, 1997.
50. CAUX C, MASSACRIER C, VANBERVLIET B, DUBOIS B, VAN KOOTEN C, DURAND I, AND BANCHEREAU J. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med* 180: 1263–1272, 1994.
51. CAUX C, VANBERVLIET B, MASSACRIER C, DEZUTTER-DAMBUYANT C, DE SAINT-VIS B, JACQUET C, YONEDA K, IMAMURA S, SCHMITT D, AND BANCHEREAU J. CD34<sup>+</sup> hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF + TNF  $\alpha$ . *J Exp Med* 184: 695–706, 1996.
52. CELLA M, ENGERING A, PINET V, PIETERS J, AND LANZAVECCHIA A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 388: 782–787, 1997.
53. CELLA M, FACCHETTI F, LANZAVECCHIA A, AND COLONNA M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* 1: 305–310, 2000.
54. CELLA M, JARROSSAY D, FACCHETTI F, ALEBARDI O, NAKAJIMA H, LANZAVECCHIA A, AND COLONNA M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 5: 919–923, 1999.
55. CELLA M, SALIO M, SAKAKIBARA Y, LANGEN H, JULKUNEN I, AND LANZAVECCHIA A. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. *J Exp Med* 189: 821–829, 1999.
56. CELLA M, SCHEIDEGGER D, PALMER-LEHMANN K, LANE P, LANZAVECCHIA A, AND ALBER G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 184: 747–752, 1996.
57. CERIO R, GRIFFITHS CE, COOPER KD, NICKOLOFF BJ, AND HEADINGTON JT. Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 121: 421–431, 1989.
58. CHAPOVAL AI, NI J, LAU JS, WILCOX RA, FLIES DB, LIU D, DONG H, SICA GL, ZHU G, TAMADA K, AND CHEN L. B7–H3: a costimulatory molecule for T cell activation and IFN- $\gamma$  production. *Nat Immunol* 2: 269–274, 2001.
59. CHEN AI, MCADAM AJ, BUHLMANN JE, SCOTT S, LUPHER, ML JR, GREENFIELD EA, BAUM PR, FANSLAW WC, CALDERHEAD DM, FREEMAN GJ, AND SHARPE AH. Ox40-ligand has a critical costimulatory role in dendritic cell: T cell interactions. *Immunity* 11: 689–698, 1999.
60. CHIU YH, JAYAWARDENA J, WEISS A, LEE D, PARK SH, DAUTRY-VARSAAT A, AND BENDELAC A. Distinct subsets of CD1d-restricted T cells recognize self-antigens loaded in different cellular compartments. *J Exp Med* 189: 103–110, 1999.
61. CHOUGNET C, FREITAG C, SCHITO M, THOMAS EK, SHER A, AND SHEARER GM. In vivo CD40-CD154 (CD40 ligand) interaction induces integrated HIV expression by APC in an HIV-1-transgenic mouse model. *J Immunol* 166: 3210–3217, 2001.
62. CLERICI M AND SHEARER GM. The Th1-Th2 hypothesis of HIV infection: new insights. *Immunol Today* 15: 575–581, 1994.
63. COOK DN, PROSSER DM, FORSTER R, ZHANG J, KUKLIN NA, ABBONDANZO SJ, NIU XD, CHEN SC, MANFRA DJ, WIEKOWSKI MT, SULLIVAN LM, SMITH SR, GREENBERG HB, NARULA SK, LIPP M, AND LIRA SA. CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* 12: 495–503, 2000.
64. CORINTI S, ALBANESI C, LA SALA A, PASTORE S, AND GIROLOMONI G. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J Immunol* 166: 4312–4318, 2001.
65. COSMAN D, FANGER N, AND BORGES L. Human cytomegalovirus, MHC class I and inhibitory signalling receptors: more questions than answers. *Immunol Rev* 168: 177–185, 1999.
66. COSMAN D, FANGER N, BORGES L, KUBIN M, CHIN W, PETERSON L, AND HSU ML. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity* 7: 273–282, 1997.
67. COSMAN D, MULLBERG J, SUTHERLAND CL, CHIN W, ARMITAGE R, FANSLAW W, KUBIN M, AND CHALUPNY NJ. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14: 123–133, 2001.
68. COTTREZ F, HURST SD, COFFMAN RL, AND GROUX H. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 165: 4848–4853, 2000.
69. COYLE AJ AND GUTIERREZ-RAMOS JC. The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function. *Nat Immunol* 2: 203–209, 2001.
70. CYSTER JG. Chemokines and cell migration in secondary lymphoid organs. *Science* 286: 2098–2102, 1999.
71. CYSTER JG. Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. *J Exp Med* 189: 447–450, 1999.
72. DALLAL RM AND LOTZE MT. The dendritic cell and human cancer vaccines. *Curr Opin Immunol* 12: 583–588, 2000.
73. D'AMBROSIO D, IELLEM A, BONECCHI R, MAZZEO D, SOZZANI S, MANTOVANI A, AND SINGAGLIA F. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. *J Immunol* 161: 5111–5115, 1998.
74. DARO E, PULENDRAN B, BRASEL K, TEEPE M, PETTIT D, LYNCH DH, VREMEC D, ROBB L, SHORTMAN K, MCKENNA HJ, MALISZEWSKI CR, AND MARASKOVSKY E. Polyethylene glycol-modified GM-CSF expands CD11b(high)CD11c(high) but not CD11b(low)CD11c(high) murine dendritic cells in vivo: a comparative analysis with Flt3 ligand. *J Immunol* 165: 49–58, 2000.
75. DAVID SA, SMITH MS, LOPEZ GJ, ADANY I, MUKHERJEE S, BUCH S, GOODENOW MM, AND NARAYAN O. Selective transmission of R5-tropic HIV type 1 from dendritic cells to resting CD4<sup>+</sup> T cells. *AIDS Res Hum Retroviruses* 17: 59–68, 2001.
76. DE JONG EC, VIEIRA PL, KALINSKI P, AND KAPSENBERG ML. Corticosteroids inhibit the production of inflammatory mediators in immature monocyte-derived DC and induce the development of tolerogenic DC3. *J Leukoc Biol* 66: 201–204, 1999.
77. DELNESTE Y, HERBAULT N, GALEA B, MAGISTRELLI G, BAZIN I, BONNEFOY JY, AND JEANNIN P. Vasoactive intestinal peptide synergizes with TNF- $\alpha$  in inducing human dendritic cell maturation. *J Immunol* 163: 3071–3075, 1999.
78. DEMANGEL C, BEAN AG, MARTIN E, FENG CG, KAMATH AT, AND BRITTON WJ. Protection against aerosol *Mycobacterium tuberculosis* infec-

- tion using *Mycobacterium bovis* *Bacillus Calmette Guerin*-infected dendritic cells. *Eur J Immunol* 29: 1972-1979, 1999.
79. DEMANGE C, PALENDIRA U, FENG CG, HEATH AW, BEAN AG, AND BRITTON WJ. Stimulation of dendritic cells via CD40 enhances immune responses to *Mycobacterium tuberculosis* infection. *Infect Immun* 69: 2456-2461, 2001.
  80. DEMEURE CE, TANAKA H, MATEO V, RUBIO M, DELESPESE G, AND SARFATI M. CD47 engagement inhibits cytokine production and maturation of human dendritic cells. *J Immunol* 164: 2193-2199, 2000.
  81. DEN HAAN JM, LEHAR SM, AND BEVAN MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 192: 1685-1696, 2000.
  82. DE SMEDT T, PAJAK B, MURAILLE E, LESPAGNARD L, HEINEN E, DE BAETSELIER P, URBAIN J, LEO O, AND MOSER M. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. *J Exp Med* 184: 1413-1424, 1996.
  83. DE SMEDT T, VAN MECHELEN M, DE BECKER G, URBAIN J, LEO O, AND MOSER M. Effect of interleukin-10 on dendritic cell maturation and function. *Eur J Immunol* 27: 1229-1235, 1997.
  84. DE ST GROTH BF. The evolution of self-tolerance: a new cell arises to meet the challenge of self-reactivity. *Immunol Today* 19: 448-454, 1998.
  85. DIEU MC, VANBERVLIET B, VICARI A, BRIDON JM, OLDHAM E, AIT-YAHIA S, BRIERE F, ZLOTNIK A, LEBECQUE S, AND CAUX C. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 188: 373-386, 1998.
  86. DIEU-NOSJEAN MC, MASSACRIER C, HOMEY B, VANBERVLIET B, PIN JJ, VICARI A, LEBECQUE S, DEZUTTER-DAMBUYANT C, SCHMITT D, ZLOTNIK A, AND CAUX C. Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J Exp Med* 192: 705-718, 2000.
  87. DIEU-NOSJEAN MC, VICARI A, LEBECQUE S, AND CAUX C. Regulation of dendritic cell trafficking: a process that involves the participation of selective chemokines. *J Leukoc Biol* 66: 252-262, 1999.
  88. DONG C, JUEDES A, TEMANN U, SHRESTA S, ALLISON J, RUDDLE N, AND FLAVEL R. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 409: 97-101, 2001.
  89. DONNELLY JJ, LIU MA, AND ULMER JB. Antigen presentation and DNA vaccines. *Am J Respir Crit Care Med* 162: S190-S193, 2000.
  90. D'OSTIANI CF, DEL SERO G, BACCI A, MONTAGNOLI C, SPRECA A, MENCACCI A, RICCIARDI-CASTAGNOLI P, AND ROMANI L. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity in vitro and in vivo. *J Exp Med* 191: 1661-1674, 2000.
  91. DREHER D, KOK M, COCHAND L, KIAMA SG, GEHR P, PECHERE JC, AND NICOD LP. Genetic background of attenuated *Salmonella typhimurium* has profound influence on infection and cytokine patterns in human dendritic cells. *J Leukoc Biol* 69: 583-589, 2001.
  92. DUBOIS B, BARTHELEMY C, DURAND I, LIU YJ, CAUX C, AND BRIERE F. Toward a role of dendritic cells in the germinal center reaction: triggering of B cell proliferation and isotype switching. *J Immunol* 162: 3428-3436, 1999.
  93. DUBOIS B, BRIDON JM, FAYETTE J, BARTHELEMY C, BANCHEREAU J, CAUX C, AND BRIERE F. Dendritic cells directly modulate B cell growth and differentiation. *J Leukoc Biol* 66: 224-230, 1999.
  94. DUBOIS B, MASSACRIER C, VANBERVLIET B, FAYETTE J, BRIERE F, BANCHEREAU J, AND CAUX C. Critical role of IL-12 in dendritic cell-induced differentiation of naive B lymphocytes. *J Immunol* 161: 2223-2231, 1998.
  95. ERB KJ, HOLLOWAY JW, SOBECK A, MOLL H, AND LE GROS G. Infection of mice with *Mycobacterium bovis*-*Bacillus Calmette-Guerin* (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med* 187: 561-569, 1998.
  96. ESCHÉ C, LOKSHIN A, SHURIN GV, GASTMAN BR, RABINOWICH H, WATKINS SC, LOTZE MT, AND SHURIN MR. Tumor's other immune targets: dendritic cells. *J Leukoc Biol* 66: 336-344, 1999.
  97. ESCHÉ C, SHURIN GV, KIRKWOOD JM, WANG GQ, RABINOWICH H, PIRTSKHALAISHVILI G, AND SHURIN MR. Tumor necrosis factor-alpha-promoted expression of Bcl-2 and inhibition of mitochondrial cytochrome c release mediate resistance of mature dendritic cells to melanoma-induced apoptosis. *Clin Cancer Res* 7: 974s-979s, 2001.
  98. FACCHETTI F, CANDIAGO E, AND VERMI W. Plasmacytoid monocytes express IL3-receptor alpha and differentiate into dendritic cells. *Histopathology* 35: 88-89, 1999.
  99. FAIRCHILD PJ AND WALDMANN H. Dendritic cells and prospects for transplantation tolerance. *Curr Opin Immunol* 12: 528-535, 2000.
  100. FAKHER M, WU J, QIN D, SZAKAL A, AND TEW J. Follicular dendritic cell accessory activity crosses MHC and species barriers. *Eur J Immunol* 31: 176-185, 2001.
  101. FANGER NA, MALISZEWSKI CR, SCHOOLEY K, AND GRIFFITH TS. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J Exp Med* 190: 1155-1164, 1999.
  102. FENG CG, DEMANGE C, KAMATH AT, MACDONALD M, AND BRITTON WJ. Dendritic cells infected with *Mycobacterium bovis* *bacillus Calmette Guerin* activate CD8(+) T cells with specificity for a novel mycobacterial epitope. *Int Immunol* 13: 451-458, 2001.
  103. FISCHER HG AND REICHMANN G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 166: 2717-2726, 2001.
  104. FLOHE SB, BAUER C, FLOHE S, AND MOLL H. Antigen-pulsed epidermal Langerhans cells protect susceptible mice from infection with the intracellular parasite *Leishmania major*. *Eur J Immunol* 28: 3800-3811, 1998.
  105. FONG L, BROCKSTEDT D, BENIKE C, WU L, AND ENGLEMAN EG. Dendritic cells injected via different routes induce immunity in cancer patients. *J Immunol* 166: 4254-4259, 2001.
  106. FONG L AND ENGLEMAN EG. Dendritic cells in cancer immunotherapy. *Annu Rev Immunol* 18: 245-273, 2000.
  107. FORSTER R, SCHUBEL A, BREITFELD D, KREMMER E, RENNER-MULLER I, WOLF E, AND LIPP M. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99: 23-33, 1999.
  108. FOTI M, GRANUCCI F, AGGUGIARO D, LIBOI E, LUINI W, MINARDI S, MANTOVANI A, SOZZANI S, AND RICCIARDI-CASTAGNOLI P. Upon dendritic cell (DC) activation chemokines and chemokine receptor expression are rapidly regulated for recruitment and maintenance of DC at the inflammatory site. *Int Immunol* 11: 979-986, 1999.
  109. FU F, LI Y, QIAN S, LU L, CHAMBERS F, STARZL TE, FUNG JJ, AND THOMSON AW. Costimulatory molecule-deficient dendritic cell progenitors (MHC class II+, CD80dim, CD86-) prolong cardiac allograft survival in nonimmunosuppressed recipients. *Transplantation* 62: 659-665, 1996.
  110. FUGIER-VIVIER I, SERVET-DELPRAT C, RIVAILLER P, RISSOAN MC, LIU YJ, AND RABOURDIN-COMBE C. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. *J Exp Med* 186: 813-823, 1997.
  111. GALLUCCI S, LOKKEMA M, AND MATZINGER P. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 5: 1249-1255, 1999.
  112. GALLUCCI S AND MATZINGER P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 13: 114-119, 2001.
  113. GAO Y, HERNDON JM, ZHANG H, GRIFFITH TS, AND FERGUSON TA. Anti-inflammatory effects of CD95 ligand (FasL)-induced apoptosis. *J Exp Med* 188: 887-896, 1998.
  114. GASCHET J, LIM A, LIEM L, VIVIER R, HALLET MM, HAROUSSEAU JL, EVEN J, GOULMY E, BONNEVILLE M, MILPIED N, AND VIE H. Acute graft versus host disease due to T lymphocytes recognizing a single HLA-DPB1\*0501 mismatch. *J Clin Invest* 98: 100-107, 1996.
  115. GELTENBEEK TB, TORENSMA R, VAN VLIET SJ, VAN DULINHOVEN GC, ADEMA GJ, VAN KOOYK Y, AND FIGDOR CG. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100: 575-585, 2000.
  116. GELTENBEEK TBH, KROOSHOOPE DJEB, BLEIS DA, VAN VLIET SJ, VAN DULINHOVEN GCF, GRABOVSKY V, ALON R, FIGDOR CG, AND VAN KOOYK Y. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* 1: 353-357, 2000.
  117. GEISSMANN F, LAUNAY P, PASQUIER B, LEPELLETIER Y, LEBORGNE M, LEHUEU A, BROUSSE N, AND MONTEIRO RC. A subset of human dendritic cells expresses IgA Fc receptor (CD89), which mediates internalization and activation upon cross-linking by IgA complexes. *J Immunol* 166: 346-352, 2001.
  118. GEORGE JF, SWEENEY SD, KIRKLIN JK, SIMPSON EM, GOLDSTEIN DR,

- AND THOMAS JM. An essential role for Fas ligand in transplantation tolerance induced by donor bone marrow. *Nat Med* 4: 333–335, 1998.
119. GILDEA LA, MORRIS RE, AND NEWMAN SL. *Histoplasma capsulatum* yeasts are phagocytosed via very late antigen-5, killed, and processed for antigen presentation by human dendritic cells. *J Immunol* 166: 1049–1056, 2001.
  120. GOLENBOCK DT AND FENTON MJ. Extolling the diversity of bacterial endotoxins. *Nat Immunol* 2: 286–288, 2001.
  121. GONG JL, MCCARTHY KM, TELFORD J, TAMATANI T, MIYASAKA M, AND SCHNEEBERGER EE. Intraepithelial airway dendritic cells: a distinct subset of pulmonary dendritic cells obtained by microdissection. *J Exp Med* 175: 797–807, 1992.
  122. GORAK PM, ENGERDA CR, AND KAYE PM. Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection. *Eur J Immunol* 28: 687–695, 1998.
  123. GOULD DS AND AUCHINCLOSS H JR. Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today* 20: 77–82, 1999.
  124. GRAKOU A, BROMLEY SK, SUMEN C, DAVIS MM, SHAW AS, ALLEN PM, AND DUSTIN ML. The immunological synapse: a molecular machine controlling T cell activation. *Science* 285: 221–227, 1999.
  125. GROHMANN U, BIANCHI R, BELLADONNA ML, VACCA C, SILLA S, AYROLDI E, FIORETTI MC, AND PUCCETTI P. IL-12 acts selectively on CD8 alpha-dendritic cells to enhance presentation of a tumor peptide in vivo. *J Immunol* 163: 3100–3105, 1999.
  126. GROUARD G, DURAND I, FILGUEIRA L, BANCHEREAU J, AND LIU YJ. Dendritic cells capable of stimulating T cells in germinal centres. *Nature* 384: 364–367, 1996.
  127. GROUARD G, RISSOAN MC, FILGUEIRA L, DURAND I, BANCHEREAU J, AND LIU YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* 185: 1101–1111, 1997.
  128. GROUX H, O'GARRA A, BIGLER M, ROULEAU M, ANTONENKO S, DE VRIES JE, AND RONCAROLO MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389: 737–742, 1997.
  129. GUERRIERO A, LANGMUIR PB, SPAIN LM, AND SCOTT EW. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. *Blood* 95: 879–885, 2000.
  130. GUNN MD, TANGEMANN K, TAM C, CYSTER JG, ROSEN SD, AND WILLIAMS LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci USA* 95: 258–263, 1998.
  131. GUNZER M, SCHAFER A, BORGMANN S, GRABBE S, ZANKER KS, BROCKER EB, KAMPGEN E, AND FRIEDL P. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity* 13: 323–332, 2000.
  132. HAHN G, JORES R, AND MOCARSKI ES. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. *Proc Natl Acad Sci USA* 95: 3937–3942, 1998.
  133. HARSHYNE LA, WATKINS SC, GAMBOTTO A, AND BARRATT-BOYES SM. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *J Immunol* 166: 3717–3723, 2001.
  134. HART DN. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* 90: 3245–3287, 1997.
  135. HERTZ C, KIERTSCHER S, GODOWSKI P, BOUIS D, NORGARD M, ROTH M, AND MODLIN R. Microbial lipopeptides stimulate dendritic cell maturation via toll-like receptor 2. *J Immunol* 166: 2444–2450, 2001.
  136. HERZ U, GERHOLD K, GRUBER C, BRAUN A, WAHN U, RENZ H, AND PAUL K. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. *J Allergy Clin Immunol* 102: 867–874, 1998.
  137. HEUFELER C, KOCH F, STANZL U, TOPAR G, WYSOCKA M, TRINCIERI G, ENK A, STEINMAN RM, ROMANI N, AND SCHULER G. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. *Eur J Immunol* 26: 659–668, 1996.
  138. HILKENS CM, KALINSKI P, DE BOER M, AND KAPSENBERG ML. Human dendritic cells require exogenous interleukin-12-inducing factors to direct the development of naive T-helper cells toward the Th1 phenotype. *Blood* 90: 1920–1926, 1997.
  139. HJELMSTROM P, JUEDES AE, FJELL J, AND RUDDLE NH. B-cell-deficient mice develop experimental allergic encephalomyelitis with demyelination after myelin oligodendrocyte glycoprotein sensitization. *J Immunol* 161: 4480–4483, 1998.
  140. HO LJ, WANG JJ, SHAO MF, KAO CL, CHANG DM, HAN SW, AND LAI JH. Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. *J Immunol* 166: 1499–1506, 2001.
  141. HOLT PG. Macrophage: dendritic cell interaction in regulation of IgE response in asthma. *Clin Exp Allergy* 23: 4–6, 1993.
  142. HOLT PG, HAINING S, NELSON DJ, AND SEDGWICK JD. Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *J Immunol* 153: 256–261, 1994.
  143. HOMEY B AND ZLOTNIK A. Chemokines in allergy. *Curr Opin Immunol* 11: 626–634, 1999.
  144. HOWIE S, RAMAGE R, AND HEWSON T. Innate immune system damage in human immunodeficiency virus type 1 infection. Implications for acquired immunity and vaccine design. *Am J Respir Crit Care Med* 162: S141–S145, 2000.
  145. HOYNE GF, TAN K, CORSIN-JIMENEZ M, WAHL K, STEWART M, HOWIE SE, AND LAMB JR. Immunological tolerance to inhaled antigen. *Am J Respir Crit Care Med* 162: S169–S174, 2000.
  146. HUANG YM, YANG JS, XU LY, LINK H, AND XIAO BG. Autoantigen-pulsed dendritic cells induce tolerance to experimental allergic encephalomyelitis (EAE) in Lewis rats. *Clin Exp Immunol* 122: 437–444, 2000.
  147. HUTLOFF A, DITTRICH AM, BEIER KC, ELIASCHEWITSCH B, KRAFT R, ANAGNOSTOPOULOS I, AND KROCZEK RA. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 397: 263–266, 1999.
  148. IEZZI G, KARJALAINEN K, AND LANZAVECCHIA A. The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* 8: 89–95, 1998.
  149. IEZZI G, SCOTET E, SCHEIDEGGER D, AND LANZAVECCHIA A. The interplay between the duration of TCR and cytokine signaling determines T cell polarization. *Eur J Immunol* 29: 4092–4101, 1999.
  150. INABA K, INABA M, DEGUCHI M, HAGI K, YASUMIZU R, IKEHARA S, MURAMATSU S, AND STEINMAN RM. Granulocytes, macrophages, and dendritic cells arise from a common major histocompatibility complex class II-negative progenitor in mouse bone marrow. *Proc Natl Acad Sci USA* 90: 3038–3042, 1993.
  151. INABA K, INABA M, ROMANI N, AYA H, DEGUCHI M, IKEHARA S, MURAMATSU S, AND STEINMAN RM. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 176: 1693–1702, 1992.
  152. INABA K, TURLEY S, IYODA T, YAMAIDE F, SHIMOYAMA S, REIS E SOUSA C, GERMAIN RN, MELLMAN I, AND STEINMAN RM. The formation of immunogenic major histocompatibility complex class II: peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J Exp Med* 191: 927–936, 2000.
  153. INGULLI E, MONDINO A, KHORUTS A, AND JENKINS MK. In vivo detection of dendritic cell antigen presentation to CD4(+) T cells. *J Exp Med* 185: 2133–2141, 1997.
  154. ITO T, INABA M, INABA K, TOKI J, SOGO S, IGUCHI T, ADACHI Y, YAMAGUCHI K, AMAKAWA R, VALLADEAU J, SAELAND S, FUKUHARA S, AND IKEHARA S. A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J Immunol* 163: 1409–1419, 1999.
  155. IWASAKI A AND KELSALL BL. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J Exp Med* 191: 1381–1394, 2000.
  156. IWASAKI A AND KELSALL BL. Unique functions of CD11b(+), CD8alpha(+), and double-negative Peyer's patch dendritic cells. *J Immunol* 166: 4884–4890, 2001.
  157. JACOB J AND BALTIMORE D. Modelling T-cell memory by genetic marking of memory T cells in vivo. *Nature* 399: 593–597, 1999.
  158. JAGER D, JAGER E, AND KNUTH A. Vaccination for malignant melanoma: recent developments. *Oncology* 60: 1–7, 2001.
  159. JAMESON SC AND BEVAN MJ. T cell receptor antagonists and partial agonists. *Immunity* 2: 1–11, 1995.
  160. JOHANSSON B, INGVARSSON S, BJORCK P, AND BORREBAECK CA. Human

- interdigitating dendritic cells induce isotype switching and IL-13-dependent IgM production in CD40-activated naive B cells. *J Immunol* 164: 1847–1854, 2000.
161. JONULEIT H, WIEDEMANN K, MULLER G, DEGWER T, HOPPE U, KNOP J, AND ENK AH. Induction of IL-15 messenger RNA and protein in human blood-derived dendritic cells: a role for IL-15 in attraction of T cells. *J Immunol* 158: 2610–2615, 1997.
  162. JOSIEN R, HESLAN M, BROUARD S, SOULILLLOU JP, AND CUTURI MC. Critical requirement for graft passenger leukocytes in allograft tolerance induced by donor blood transfusion. *Blood* 92: 4539–4544, 1998.
  163. JOSIEN R, WONG BR, LI HL, STEINMAN RM, AND CHOI Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. *J Immunol* 162: 2562–2568, 1999.
  164. JUEDES AE AND RUDDLE NH. Resident and infiltrating central nervous system APCs regulate the emergence and resolution of experimental autoimmune encephalomyelitis. *J Immunol* 166: 5168–5175, 2001.
  165. KADOWAKI N, ANTONENKO S, LAU JY, AND LIU YJ. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J Exp Med* 192: 219–226, 2000.
  166. KAISHO T AND AKIRA S. Dendritic-cell function in Toll-like receptor- and MyD88-knockout mice. *Trends Immunol* 22: 78–83, 2001.
  167. KALINSKI P, HILKENS CM, WIERENGA EA, SNLDEWINT FG, AND KAPSENBERG ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E<sub>2</sub>, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* 159: 28–35, 1997.
  168. KALINSKI P, HILKENS CM, WIERENGA EA, AND KAPSENBERG ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today* 20: 561–567, 1999.
  169. KALINSKI P, SCHUITEMAKER JH, HILKENS CM, AND KAPSENBERG ML. Prostaglandin E<sub>2</sub> induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol* 161: 2804–2809, 1998.
  170. KAMATH AT, POOLEY J, O'KEEFFE MA, VREMEC D, ZHAN Y, LEW AM, D'AMICO A, WU L, TOUGH DF, AND SHORTMAN K. The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J Immunol* 165: 6762–6770, 2000.
  171. KAWAMURA T AND FURUE M. Comparative analysis of B7-1 and B7-2 expression in Langerhans cells: differential regulation by T helper type 1 and T helper type 2 cytokines. *Eur J Immunol* 25: 1913–1917, 1995.
  172. KHOURY SJ, GALLON L, VERBURG RR, CHANDRAKER A, PEACH R, LINSLEY PS, TURKA LA, HANCOCK WW, AND SAYEGH MH. Ex vivo treatment of antigen-presenting cells with CTLA4Ig and encephalitogenic peptide prevents experimental autoimmune encephalomyelitis in the Lewis rat. *J Immunol* 157: 3700–3705, 1996.
  173. KO S, DEWICK A, JAGER MD, DINKEL A, ROHDE F, FISCHER R, TSUI TY, RITTMANN KL, WONGEIT K, AND SCHLIT HJ. The functional relevance of passenger leukocytes and microchimerism for heart allograft acceptance in the rat. *Nat Med* 5: 1292–1297, 1999.
  174. KOHRGRUBER N, HALANEK N, GROGER M, WINTER D, RAPPERSBERGER K, SCHMITT-EGENOLF M, STINGL G, AND MAURER D. Survival, maturation, and function of CD11c- and CD11c+ peripheral blood dendritic cells are differentially regulated by cytokines. *J Immunol* 163: 3250–3259, 1999.
  175. KOPF M, COYLE AJ, SCHMITZ N, BARNER M, OXENIUS A, GALLIMORE A, GUTIERREZ-RAMOS JC, AND BACHMANN MF. Inducible costimulator protein (ICOS) controls T helper cell subset polarization after virus and parasite infection. *J Exp Med* 192: 53–61, 2000.
  176. KOPF M, RUEDL C, SCHMITZ N, GALLIMORE A, LEFRANG K, ECABERT B, ODERMATT B, AND BACHMANN MF. OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL responses after virus infection. *Immunity* 11: 699–708, 1999.
  177. KORPI M, KUIKKA L, REIJONEN T, REMES K, JUNTUNEN-BACKMAN K, AND LAUNIALA K. Bronchial asthma and hyperreactivity after early childhood bronchiolitis or pneumonia. *Arch Pediatr Adolesc Med* 148: 1079–1084, 1994.
  178. KRONIN V, VREMEC D, AND SHORTMAN K. Does the IL-2 receptor alpha chain induced on dendritic cells have a biological function? *Int Immunol* 10: 237–240, 1998.
  179. KRONIN V, WINKEL K, SUSS G, KELSO A, HEATH W, KIRBERG J, VON BOEHMER H, AND SHORTMAN K. A subclass of dendritic cells regulates the response of naive CD8 T cells by limiting their IL-2 production. *J Immunol* 157: 3819–3827, 1996.
  180. KRONIN V, WU L, GONG S, NUSSENZWEIG MC, AND SHORTMAN K. DEC-205 as a marker of dendritic cells with regulatory effects on CD8 T cell responses. *Int Immunol* 12: 731–735, 2000.
  181. KU CC, MURAKAMI M, SAKAMOTO A, KAPPLER J, AND MARRACK P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. *Science* 288: 675–678, 2000.
  182. KUNKEL SL. Through the looking glass: the diverse in vivo activities of chemokines. *J Clin Invest* 104: 1333–1334, 1999.
  183. KURODA E, SUGIURA T, ZEKI K, YOSHIDA Y, AND YAMASHITA U. Sensitivity difference to the suppressive effect of prostaglandin E<sub>2</sub> among mouse strains: a possible mechanism to polarize Th2 type response in BALB/c mice. *J Immunol* 164: 2386–2395, 2000.
  184. KURTS C, CANNARILE M, KLEBBA I, AND BROCKER T. Dendritic cells are sufficient to cross-present self-antigens to CD8 T cells in vivo. *J Immunol* 166: 1439–1442, 2001.
  185. LAMBRECHT BN, SALOMON B, KLATZMANN D, AND PAUWELS RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J Immunol* 160: 4090–4097, 1998.
  186. LANGENKAMP A, MESSI M, LANZAVECCHIA A, AND SALLUSTO F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nature Immunol* 1: 311–316, 2000.
  187. LANZAVECCHIA A, LEZZI G, AND VIOLA A. From TCR engagement to T cell activation: a kinetic view of T cell behavior. *Cell* 96: 1–4, 1999.
  188. LANZAVECCHIA A AND SALLUSTO F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science* 290: 92–97, 2000.
  189. LANZAVECCHIA A AND SALLUSTO F. From synapses to immunological memory: the role of sustained T cell stimulation. *Curr Opin Immunol* 12: 92–98, 2000.
  190. LARSEN CP, MORRIS PJ, AND AUSTYN JM. Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. *J Exp Med* 171: 307–314, 1990.
  191. LARSSON M, FONTENEAU JF, AND BHARDWAJ N. Dendritic cells resurface antigens from dead cells. *Trends Immunol* 22: 141–148, 2001.
  192. LAU LL, JAMIESON BD, SOMASUNDARAM T, AND AHMED R. Cytotoxic T-cell memory without antigen. *Nature* 369: 648–652, 1994.
  193. LEE JI, GANSTER RW, GELLER DA, BURCKART GJ, THOMSON AW, AND LU L. Cyclosporine A inhibits the expression of costimulatory molecules on in vitro-generated dendritic cells: association with reduced nuclear translocation of nuclear factor kappa B. *Transplantation* 68: 1255–1263, 1999.
  194. LEE WC, ZHONG C, QIAN S, WAN Y, GAULDIE J, MI Z, ROBBINS PD, THOMSON AW, AND LU L. Phenotype, function, and in vivo migration and survival of allogeneic dendritic cell progenitors genetically engineered to express TGF-beta. *Transplantation* 66: 1810–1817, 1998.
  195. LEENEN PJ, RADOSEVIC K, VOERMAN JS, SALOMON B, VAN ROOIJEN N, KLATZMANN D, AND VAN EWLIK W. Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover. *J Immunol* 160: 2166–2173, 1998.
  196. LI Y, LI XC, ZHENG XX, WELLS AD, TURKA LA, AND STROM TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat Med* 5: 1298–1302, 1999.
  197. LIAU LM, BLACK KL, PRINS RM, SYKES SN, DIPATRE PL, CLOUGHESY TF, BECKER DP, AND BRONSTEIN JM. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90: 1115–1124, 1999.
  198. LIN CL, SURI RM, RAHDON RA, AUSTYN JM, AND ROAKE JA. Dendritic cell chemotaxis and transendothelial migration are induced by distinct chemokines and are regulated on maturation. *Eur J Immunol* 28: 4114–4122, 1998.
  199. LINDHOUT E, VISSERS JL, HARTGERS FC, HULBENS RJ, SCHARENBERG NM, FIGDOR CG, AND ADEMA GJ. The dendritic cell-specific chemokine dc-ck1 is expressed by germinal center dendritic cells and attracts cd38-negative mantle zone B lymphocytes. *J Immunol* 166: 3284–3289, 2001.

200. LINK H, HUANG YM, MASTERMAN T, AND XIAO BG. Vaccination with autologous dendritic cells: from experimental autoimmune encephalomyelitis to multiple sclerosis. *J Neuroimmunol* 114: 1-7, 2001.
201. LINK H, HUANG YM, AND XIAO BG. Dendritic cells in experimental allergic encephalomyelitis and multiple sclerosis. *J Neuroimmunol* 100: 102-110, 1999.
202. LIPSCOMB MF, BICE DE, LYONS CR, SCHUYLER MR, AND WILKES D. The regulation of pulmonary immunity. *Adv Immunol* 59: 369-455, 1995.
203. LIPSCOMB MF, POLLARD AM, AND YATES JL. A role for TGF-beta in the suppression by murine bronchoalveolar cells of lung dendritic cell initiated immune responses. *Reg Immunol* 5: 151-157, 1993.
204. LIPSCOMB MF AND WILDER JA. Immune dysregulation as a cause for allergic asthma. *Curr Opin Pulmon Med* 5: 10-20, 1999.
205. LIU L, RICH BE, INOBE J, CHEN W, AND WEINER HL. A potential pathway of Th2 development during primary immune response. IL-10 pretreated dendritic cells can prime naive CD4+ T cells to secrete IL-4. *Adv Exp Med Biol* 417: 375-381, 1997.
206. LOENEN WA, BRUGGEMAN CA, AND WIERTZ EJ. Immune evasion by human cytomegalovirus: lessons in immunology and cell biology. *Semin Immunol* 13: 41-49, 2001.
207. LU L, GAMBOTTO A, LEE WC, QIAN S, BONHAM CA, ROBBINS PD, AND THOMSON AW. Adenoviral delivery of CTLA4Ig into myeloid dendritic cells promotes their in vitro tolerogenicity and survival in allogeneic recipients. *Gene Ther* 6: 554-563, 1999.
208. LU L, LEE WC, TAKAYAMA T, QIAN S, GAMBOTTO A, ROBBINS PD, AND THOMSON AW. Genetic engineering of dendritic cells to express immunosuppressive molecules (viral IL-10, TGF-beta, and CTLA4Ig). *J Leukoc Biol* 66: 293-296, 1999.
209. LU L, LI W, FU F, CHAMBERS FG, QIAN S, FUNG JJ, AND THOMSON AW. Blockade of the CD40-CD40 ligand pathway potentiates the capacity of donor-derived dendritic cell progenitors to induce long-term cardiac allograft survival. *Transplantation* 64: 1808-1815, 1997.
210. LU L, LI W, ZHONG C, QIAN S, FUNG JJ, THOMSON AW, AND STARZL TE. Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation* 68: 747-757, 1999.
211. LU L, MCCASLIN D, STARZL TE, AND THOMSON AW. Bone marrow-derived dendritic cell progenitors (NLDC 145+, MHC class II+, B7-1 dim, B7-2-) induce alloantigen-specific hyporesponsiveness in murine T lymphocytes. *Transplantation* 60: 1539-1545, 1995.
212. LU L, QIAN S, HERSHBERGER PA, RUDERT WA, LYNCH DH, AND THOMSON AW. Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. *J Immunol* 158: 5676-5684, 1997.
213. LU L, RUDERT WA, QIAN S, MCCASLIN D, FU F, RAO AS, TRUCCO M, FUNG JJ, STARZL TE, AND THOMSON AW. Growth of donor-derived dendritic cells from the bone marrow of murine liver allograft recipients in response to granulocyte/macrophage colony-stimulating factor. *J Exp Med* 182: 379-387, 1995.
214. LUDEWIG B, OCHSENBEIN AF, ODERMATT B, PAULIN D, HENGARTNER H, AND ZINKERNAGEL RM. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J Exp Med* 191: 795-804, 2000.
215. LUDEWIG B, ODERMATT B, OCHSENBEIN AF, ZINKERNAGEL RM, AND HENGARTNER H. Role of dendritic cells in the induction and maintenance of autoimmune diseases. *Immunol Rev* 169: 45-54, 1999.
216. LUGTON I. Mucosa-associated lymphoid tissues as sites for uptake, carriage and excretion of tubercle bacilli and other pathogenic mycobacteria. *Immunol Cell Biol* 77: 364-372, 1999.
217. MACKENSEN A, HERBST B, CHEN JL, KOHLER G, NOPPEN C, HERR W, SPAGNOLI GC, CERUNDOLO V, AND LINDEMANN A. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. *Int J Cancer* 86: 385-392, 2000.
218. MALDONADO-LOPEZ R, DE SMEDT T, MICHEL P, GODFROID J, PAJAK B, HEIRMAN C, THIELEMANS K, LEO O, URBAIN J, AND MOSER M. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med* 189: 587-592, 1999.
219. MALDONADO-LOPEZ R, DE SMEDT T, PAJAK B, HEIRMAN C, THIELEMANS K, LEO O, URBAIN J, MALISZEWSKI CR, AND MOSER M. Role of CD8alpha+ and CD8alpha- dendritic cells in the induction of primary immune responses in vivo. *J Leukoc Biol* 66: 242-246, 1999.
220. MANDELBROT DA, FURUKAWA Y, MCADAM AJ, ALEXANDER SI, LIBBY P, MITCHELL RN, AND SHARPE AH. Expression of B7 molecules in recipient, not donor, mice determines the survival of cardiac allografts. *J Immunol* 163: 3753-3757, 1999.
221. MARASKOVSKY E, BRASEL K, TEEPE M, ROUX ER, LYMAN SD, SHORTMAN K, AND MCKENNA HJ. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med* 184: 1953-1962, 1996.
222. MARASKOVSKY E, DARO E, ROUX E, TEEPE M, MALISZEWSKI CR, HOEK J, CARON D, LEBACK ME, AND MCKENNA HJ. In vivo generation of human dendritic cell subsets by Flt3 ligand. *Blood* 96: 878-884, 2000.
223. MARIE JC, KEHREN J, TRESOL-BIEMONT MC, EVLASHEV A, VALENTIN H, WALZER T, TEDONE R, LOVELAND B, NICOLAS JF, RABOURDIN-COMBE C, AND HORVAT B. Mechanism of measles virus-induced suppression of inflammatory immune responses. *Immunity* 14: 69-79, 2001.
224. MAROVICH MA, McDOWELL MA, THOMAS EK, AND NUTMAN TB. IL-12p70 production by Leishmania major-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. *J Immunol* 164: 5858-5865, 2000.
225. MARRIOTT I, HAMMOND TG, THOMAS EK, AND BOST KL. *Salmonella* efficiently enter and survive within cultured CD11c+ dendritic cells initiating cytokine expression. *Eur J Immunol* 29: 1107-1115, 1999.
226. MARTIN P, DEL HOYO GM, ANJUERE F, RUIZ SR, ARIAS CF, MARIN AR, AND ARDAVIN C. Concept of lymphoid versus myeloid dendritic cell lineages revisited: both CD8alpha(-) and CD8alpha(+) dendritic cells are generated from CD4(low) lymphoid-committed precursors. *Blood* 96: 2511-2519, 2000.
227. MASTEN BJ AND LIPSCOMB MF. Methods to isolate and study lung dendritic cells. In: *Lung Macrophages and Dendritic Cells*, edited by Lipscomb MF and Russell SW. New York: Dekker, 1997, p. 223-238.
228. MASTEN BJ AND LIPSCOMB MF. Comparison of lung dendritic cells and B cells in stimulating naive antigen-specific T cells. *J Immunol* 162: 1310-1317, 1999.
229. MASTEN BJ, YATES JL, POLLARD KOGA AM, AND LIPSCOMB MF. Characterization of accessory molecules in murine lung dendritic cell function: roles for CD80, CD86, CD54, and CD40L. *Am J Respir Cell Mol Biol* 16: 335-342, 1997.
230. MATSUE H, MATSUE K, WALTERS M, OKUMURA K, YAGITA H, AND TAKASHIMA A. Induction of antigen-specific immunosuppression by CD95L cDNA-transfected "killer" dendritic cells. *Nat Med* 5: 930-937, 1999.
231. MATZINGER P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 12: 991-1045, 1994.
232. MCADAM A, GREENWALD RJ, LEVIN MA, CHERNOVA T, MALENKOVICH N, LING V, FREEMAN GJ, AND SHARPE AH. ICOS is critical for CD40-mediated antibody class switching. *Nature* 409: 102-105, 2001.
233. MCWILLIAM AS, NELSON D, THOMAS JA, AND HOLT PG. Rapid dendritic cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. *J Exp Med* 179: 1331-1336, 1994.
234. MEDZHTOV R AND JANEWAY C JR. Innate immunity. *N Engl J Med* 343: 338-344, 2000.
235. MILLER MD AND KRANGEL MS. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* 12: 17-46, 1992.
236. MIN WP, GORCZYNSKI R, HUANG XY, KUSHIDA M, KIM P, OBATAKI M, LEI J, SURI RM, AND CATRAL MS. Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. *J Immunol* 164: 161-167, 2000.
237. MOLLER GM, OVERBEEK SE, VAN HELDEN-MEEUWSEN CG, VAN HAARST JM, PRENS EP, MULDER PG, POSTMA DS, AND HOOGSTEDEN HC. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. *Clin Exp Allergy* 26: 517-524, 1996.
238. MOORE KW, O'GARRA A, DE WAAL MALEYFYT R, VIEIRA P, AND MOSMANN TR. Interleukin-10. *Annu Rev Immunol* 11: 165-190, 1993.
239. MORELLI A, LARREGINA A, CHULUYAN I, KOLKOWSKI E, AND FAINBOIM L.

- Expression and modulation of C5a receptor (CD88) on skin dendritic cells. Chemotactic effect of C5a on skin migratory dendritic cells. *Immunology* 89: 126–134, 1996.
240. MORELLI AE AND THOMSON AW. Role of dendritic cells in the immune response against allografts. *Curr Opin Nephrol Hypertens* 9: 607–613, 2000.
  241. MORRIS P, SHAMAN J, ATTAYA M, AMAYA M, GOODMAN S, BERGMAN C, MONACO JJ, AND MELLINS E. An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature* 368: 551–554, 1994.
  242. MULLER WA AND RANDOLPH GJ. Migration of leukocytes across endothelium and beyond: molecules involved in the transmigration and fate of monocytes. *J Leukoc Biol* 66: 698–704, 1999.
  243. MURPHY WJ AND BLAZAR BR. New strategies for preventing graft-versus-host disease. *Curr Opin Immunol* 11: 509–515, 1999.
  244. MUTINI C, FALZONI S, FERRARI D, CHIOZZI P, MORELLI A, BARICORDI OR, COLLO G, RICCIARDI-CASTAGNOLI P, AND DI VIRGILIO F. Mouse dendritic cells express the P2X7 purinergic receptor: characterization and possible participation in antigen presentation. *J Immunol* 163: 1958–1965, 1999.
  245. MUZIO M, BOSISIO D, POLENTARUTTI N, D'AMICO G, STOPPACCIARO A, MANCINELLI R, VAN'T VEER C, PENTON-ROL G, RUCCO LP, ALLAVENA P, AND MANTOVANI A. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J Immunol* 164: 5998–6004, 2000.
  246. NELSON WG, SIMONS JW, MIKHAK B, CHANG JF, DEMARZO AM, CARDUCCI MA, KIM M, WEBER CE, BACCALA AA, GOEMAN MA, CLIFT SM, ANDO DG, LEVITSKY HI, COHEN LK, SANDA MG, MULLIGAN RC, PARTIN AW, CARTER HB, PIANTADOSI S, AND MARSHALL FF. Cancer cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer as vaccines for the treatment of genitourinary malignancies. *Cancer Chemother Pharmacol* 46: S67–S72, 2000.
  247. NGO VN, TANG HL, AND CYSTER JG. Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. *J Exp Med* 188: 181–191, 1998.
  248. NOVAK N, HABERSTOK J, GEIGER E, AND BIEBER T. Dendritic cells in allergy. *Allergy* 54: 792–493, 1999.
  249. O'DOHERTY U, PENG M, GEZELTER S, SWIGGARD WJ, BETJES M, BHARDWAJ N, AND STEINMAN RM. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* 82: 487–493, 1994.
  250. OHASHI K, BURKART V, FLOHE S, AND KOLB H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 164: 558–561, 2000.
  251. OHTEKI T, FUKAO T, SUZUE K, MAKI C, ITO M, NAKAMURA M, AND KOYASU S. Interleukin 12-dependent interferon gamma production by CD8alpha+ lymphoid dendritic cells. *J Exp Med* 189: 1981–1986, 1999.
  252. OLIVER S, EDWARDS AD, SCHITO M, ALIBERTI J, MANICKASINGHAM S, SHER A, AND REIS E SOUSA C. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity* 13: 453–462, 2000.
  253. OWENS T AND SRIRAM S. The immunology of multiple sclerosis and its animal model, experimental allergic encephalomyelitis. *Neurol Clin* 13: 51–73, 1995.
  254. PAMER E AND CRESSWELL P. Mechanisms of MHC class I-restricted antigen processing. *Annu Rev Immunol* 16: 323–358, 1998.
  255. PATTERSON S. Flexibility and cooperation among dendritic cells. *Nat Immunol* 1: 273–274, 2000.
  256. PEGGS K, VERFUERTH S, AND MACKINNON S. Induction of cytomegalovirus (CMV)-specific T-cell responses using dendritic cells pulsed with CMV antigen: a novel culture system free of live CMV virions. *Blood* 97: 994–1000, 2001.
  257. PEGUET-NAVARRO J, MOULON C, CAUX C, DALBIEZ-GAUTHIER C, BANCHEREAU J, AND SCHMITT D. Inhibitory effect of IL-10 on human Langerhans cell antigen presenting function. *Adv Exp Med Biol* 378: 359–361, 1995.
  258. PFEIFER JD, WICK MJ, ROBERTS RL, FINDLAY K, NORMARK SJ, AND HARDING CV. Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* 361: 359–362, 1993.
  259. PIEMONTE L, MONTI P, SIRONI M, FRATICELLI P, LEONE BE, DAL CIN E, ALLAVENA P, AND DI CARLO V. Vitamin D<sub>3</sub> affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* 164: 4443–4451, 2000.
  260. PROBST P, SKEIKY YA, STEEVES M, GERVASSI A, GRABSTEIN KH, AND REED SG. A Leishmania protein that modulates interleukin (IL)-12, IL-10 and tumor necrosis factor-alpha production and expression of B7-1 in human monocyte-derived antigen-presenting cells. *Eur J Immunol* 27: 2634–2642, 1997.
  261. PRON B, BOUMAILA C, JAUBERT F, BERCHE P, MILON G, GEISSMANN F, AND GAILLARD JL. Dendritic cells are early cellular targets of Listeria monocytogenes after intestinal delivery and are involved in bacterial spread in the host. *Cell Microbiol* 3: 331–340, 2001.
  262. PULENDRAN B, BANCHEREAU J, BURKEHOLDER S, KRAUS E, GUINET E, CHALOUNI C, CARON D, MALISZEWSKI C, DAVOUST J, FAY J, AND PALUCKA K. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol* 165: 566–572, 2000.
  263. PULENDRAN B, BANCHEREAU J, MARASKOVSKY E, AND MALISZEWSKI C. Modulating the immune response with dendritic cells and their growth factors. *Trends Immunol* 22: 41–47, 2001.
  264. PULENDRAN B, LINGAPPA J, KENNEDY MK, SMITH J, TEEPE M, RUDENSKY A, MALISZEWSKI CR, AND MARASKOVSKY E. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. *J Immunol* 159: 2222–2231, 1997.
  265. PULENDRAN B, SMITH JL, CASPARY G, BRASEL K, PETTIT D, MARASKOVSKY E, AND MALISZEWSKI CR. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci USA* 96: 1036–1041, 1999.
  266. QIAN S, LU L, FU F, LI Y, LI W, STARZL TE, FUNG JJ, AND THOMSON AW. Apoptosis within spontaneously accepted mouse liver allografts: evidence for deletion of cytotoxic T cells and implications for tolerance induction. *J Immunol* 158: 4654–4661, 1997.
  267. QIN D, WU J, VORA KA, RAVETCH JV, SZAKAL AK, MANSEY T, AND TEW JG. Fc gamma receptor IIB on follicular dendritic cells regulates the B cell recall response. *J Immunol* 164: 6268–6275, 2000.
  268. RANDOLPH GJ, BEAULIEU S, LEBECQUE S, STEINMAN RM, AND MULLER WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* 282: 480–483, 1998.
  269. REID SD, PENNA G, AND ADORINI L. The control of T cell responses by dendritic cell subsets. *Curr Opin Immunol* 12: 114–121, 2000.
  270. REIS E SOUSA C AND GERMAIN RN. Major histocompatibility complex class I presentation of peptides derived from soluble exogenous antigen by a subset of cells engaged in phagocytosis. *J Exp Med* 182: 841–851, 1995.
  271. REIS E SOUSA C, SHER A, AND KAYE P. The role of dendritic cells in the induction and regulation of immunity to microbial infection. *Curr Opin Immunol* 11: 392–399, 1999.
  272. RES PC, COUWENBERG F, VYTH-DREESE FA, AND SPITS H. Expression of pTalpha mRNA in a committed dendritic cell precursor in the human thymus. *Blood* 94: 2647–2657, 1999.
  273. RESCIGNO M, GRANUCCI F, AND RICCIARDI-CASTAGNOLI P. Dendritic cells at the end of the millennium. *Immunol Cell Biol* 77: 404–410, 1999.
  274. RESCIGNO M, FIGUET V, VALZASINA B, LENS S, ZUBLER R, FRENCH L, KINDLER V, TSCHOPP J, AND RICCIARDI-CASTAGNOLI P. Fas engagement induces the maturation of dendritic cells (DCs), the release of interleukin (IL)-1beta, and the production of interferon gamma in the absence of IL-12 during DC-T cell cognate interaction: a new role for Fas ligand in inflammatory responses. *J Exp Med* 192: 1661–1668, 2000.
  275. RICHTERS CD, VAN GELDEROP E, DU PONT JS, HOEKSTRA MJ, KREIS RW, AND KAMPERDIJK EW. Migration of dendritic cells to the draining lymph node after allogeneic or congenic rat skin transplantation. *Transplantation* 67: 828–832, 1999.
  276. RIEGLER S, HEBART H, EINSELE H, BROSSART P, JAHN G, AND SINZGER C. Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. *J Gen Virol* 81: 393–399, 2000.
  277. RODRIGUEZ A, REGNAULT A, KLEIMEER M, RICCIARDI-CASTAGNOLI P, AND AMIGORENA S. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat Cell Biol* 1: 362–368, 1999.

278. ROLLINS BJ. Chemokines. *Blood* 90: 909–928, 1997.
279. ROMANI N, GRUNER S, BRANG D, KAMPGEN E, LENZ A, TROCKENBACHER B, KONWALINKA G, FRITSCH PO, STEINMAN RM, AND SCHULER G. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 180: 83–93, 1994.
280. RUEDL C AND BACHMANN MF. CTL priming by CD8(+) and CD8(–) dendritic cells in vivo. *Eur J Immunol* 29: 3762–3767, 1999.
281. RUTAULT K, ALDERMAN C, CHAIN BM, AND KATZ DR. Reactive oxygen species activate human peripheral blood dendritic cells. *Free Radical Biol Med* 26: 232–238, 1999.
282. SALLUSTO F, KREMMER E, PALERMO B, HOY A, PONATH P, QIN S, FORSTER R, LIPP M, AND LANZAVECCHIA A. Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. *Eur J Immunol* 29: 2037–2045, 1999.
283. SALLUSTO F AND LANZAVECCHIA A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179: 1109–1118, 1994.
284. SALLUSTO F AND LANZAVECCHIA A. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol Rev* 177: 134–140, 2000.
285. SALLUSTO F, LENIG D, FORSTER R, LIPP M, AND LANZAVECCHIA A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708–712, 1999.
286. SALLUSTO F, MACKAY CR, AND LANZAVECCHIA A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 277: 2005–2007, 1997.
287. SALLUSTO F, MACKAY CR, AND LANZAVECCHIA A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 18: 593–620, 2000.
288. SALLUSTO F, PALERMO B, LENIG D, MIETTINEN M, MATIKAINEN S, JULKUNEN I, FORSTER R, BURGSTALLER R, LIPP M, AND LANZAVECCHIA A. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. *Eur J Immunol* 29: 1617–1625, 1999.
289. SALLUSTO F, SCHAEERLI P, LOETSCHER P, SCHANIEL C, LENIG D, MACKAY CR, QIN S, AND LANZAVECCHIA A. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* 28: 2760–2769, 1998.
290. SALOMON B AND BLUESTONE JA. LFA-1 interaction with ICAM-1 and ICAM-2 regulates Th2 cytokine production. *J Immunol* 161: 5138–5142, 1998.
291. SALOMON B, COHEN JL, MASURIER C, AND KLATZMANN D. Three populations of mouse lymph node dendritic cells with different origins and dynamics. *J Immunol* 160: 708–717, 1998.
292. SANDBERG K, MATSSON P, AND ALM GV. A distinct population of nonphagocytic and low level CD4+ null lymphocytes produce IFN-alpha after stimulation by herpes simplex virus-infected cells. *J Immunol* 145: 1015–1020, 1990.
293. SAUNDERS D, LUCAS K, ISMAILI J, WU L, MARASKOVSKY E, DUNN A, AND SHORTMAN K. Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. *J Exp Med* 184: 2185–2196, 1996.
294. SCARLATTI G, TRESOLDI E, BJORNDAAL A, FREDRIKSSON R, COLOGNESI C, DENG HK, MALNATI MS, PLEBANI A, SICCARDI AG, LITTMAN DR, FENYO EM, AND LUSSO P. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nat Med* 3: 1259–1265, 1997.
295. SCHOPPET M AND HUPPERTZ HI. Differential stimulation of helper and cytotoxic T cells by dendritic cells after infection by *Yersinia enterocolitica* in vitro. *Cell Immunol* 208: 43–51, 2001.
296. SCHULER G AND STEINMAN RM. Dendritic cells as adjuvants for immune-mediated resistance to tumors. *J Exp Med* 186: 1183–1187, 1997.
297. SCHWARTZ RH. Immunology. It takes more than two to tango. *Nature* 409: 31–32, 2001.
298. SEMPER AE AND HARTLEY JA. Dendritic cells in the lung: what is their relevance to asthma? *Clin Exp Allergy* 26: 485–490, 1996.
299. SERAFINI B, COLUMBA-CABEZAS S, DI ROSA F, AND ALOISI F. Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am J Pathol* 157: 1991–2002, 2000.
300. SERVET-DELPRAT C, VIDALAIN PO, BAUSINGER H, MANIE S, LE DEIST F, AZOCAR O, HANAU D, FISCHER A, AND RABOURDIN-COMBE C. Measles virus induces abnormal differentiation of CD40 ligand-activated human dendritic cells. *J Immunol* 164: 1753–1760, 2000.
301. SHEDLOCK DJ AND WEINER DB. DNA vaccination: antigen presentation and the induction of immunity. *J Leukoc Biol* 68: 793–806, 2000.
302. SHI GP, BRYANT RA, RIESE R, VERHELST S, DRIESSEN C, LI Z, BROMME D, PLOEGH HL, AND CHAPMAN HA. Role for cathepsin F in invariant chain processing and major histocompatibility complex class II peptide loading by macrophages. *J Exp Med* 191: 1177–1186, 2000.
303. SHINKAI K AND LOCKSLEY RM. CD1, tuberculosis, and the evolution of major histocompatibility complex molecules. *J Exp Med* 191: 907–914, 2000.
304. SHIRAKAWA T, ENOMOTO T, SHIMAZU S, AND HOPKIN JM. The inverse association between tuberculin responses and atopic disorder. *Science* 275: 77–79, 1997.
305. SHORTMAN K. Burnet oration: dendritic cells: multiple subtypes, multiple origins, multiple functions. *Immunol Cell Biol* 78: 161–165, 2000.
306. SHU U, KINIWA M, WU CY, MALISZEWSKI C, VEZZIO N, HAKIMI J, GATELY M, AND DELESPESE G. Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. *Eur J Immunol* 25: 1125–1128, 1995.
307. SIEGAL FP, KADOWAKI N, SHODELL M, FITZGERALD-BOCARSLY PA, SHAH K, HO S, ANTONENKO S, AND LIU YJ. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284: 1835–1837, 1999.
308. SIGURS N, BJARNASON R, SIGURBERGSSON F, KJELLMAN B, AND BJORKSTEN B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 95: 500–505, 1995.
309. SINGH-JASUJA H, SCHERER HU, HILF N, ARNOLD-SCHILD D, RAMMENSEE HG, TOES RE, AND SCHILD H. The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. *Eur J Immunol* 30: 2211–2215, 2000.
310. SLOAN-LANCASTER J, EVAVOLD BD, AND ALLEN PM. Th2 cell clonal anergy as a consequence of partial activation. *J Exp Med* 180: 1195–1205, 1994.
311. SMITH KM, EATON AD, FINLAYSON LM, AND GARSIDE P. Oral tolerance. *Am J Respir Crit Care Med* 162: S175–S178, 2000.
312. SMYTH MJ, GODFREY DI, AND TRAPANI JA. A fresh look at tumor immunosurveillance and immunotherapy. *Nat Immunol* 2: 293–299, 2001.
313. SNILDERS A, KALINSKI P, HILKENS CM, AND KAPSENBERG ML. High-level IL-12 production by human dendritic cells requires two signals. *Int Immunol* 10: 1593–1598, 1998.
314. SORG RV, KOGLER G, AND WERNET P. Identification of cord blood dendritic cells as an immature CD11c– population. *Blood* 93: 2302–2307, 1999.
315. SOZZANI S, ALLAVENA P, D'AMICO G, LUINI W, BIANCHI G, KATAURA M, IMAI T, YOSHIE O, BONECCHI R, AND MANTOVANI A. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. *J Immunol* 161: 1083–1086, 1998.
316. SOZZANI S, ALLAVENA P, VECCHI A, AND MANTOVANI A. The role of chemokines in the regulation of dendritic cell trafficking. *J Leukoc Biol* 66: 1–9, 1999.
317. SOZZANI S, LONGONI D, BONECCHI R, LUINI W, BERSANI L, D'AMICO G, BORSATTI A, BUSSOLINO F, ALLAVENA P, AND MANTOVANI A. Human monocyte-derived and CD34+ cell-derived dendritic cells express functional receptors for platelet activating factor. *FEBS Lett* 418: 98–100, 1997.
318. SOZZANI S, LUINI W, BORSATTI A, POLENTARUTTI N, ZHOU D, PIEMONTE L, D'AMICO G, POWER CA, WELLS TN, GOBBI M, ALLAVENA P, AND MANTOVANI A. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J Immunol* 159: 1993–2000, 1997.
319. SOZZANI S, SALLUSTO F, LUINI W, ZHOU D, PIEMONTE L, ALLAVENA P, VAN DAMME J, VALITUTTI S, LANZAVECCHIA A, AND MANTOVANI A. Migration of dendritic cells in response to formyl peptides, C5a, and a distinct set of chemokines. *J Immunol* 155: 3292–3295, 1995.
320. SPITS H, COUWENBERG F, BAKKER AQ, WELER K, AND UITTENBOGAART

- CH. Id2 and Id3 inhibit development of CD34(+) stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. *J Exp Med* 192: 1775-1784, 2000.
321. STARZL TE, DEMETRIS AJ, MURASE N, ILSTAD S, RICORDI C, AND TRUCCO M. Cell migration, chimerism, and graft acceptance. *Lancet* 339: 1579-1582, 1992.
  322. STARZL TE, DEMETRIS AJ, TRUCCO M, MURASE N, RICORDI C, ILSTAD S, RAMOS H, TODO S, TZAKIS A, FUNG JJ, NALESNIK M, ZEEVI A, RUDERT WA, AND KOCOVA M. Cell migration and chimerism after whole-organ transplantation: the basis of graft acceptance. *Hepatology* 17: 1127-1152, 1993.
  323. STEINBRINK K, WOLFEL M, JONULEIT H, KNOP J, AND ENK AH. Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 159: 4772-4780, 1997.
  324. STEINMAN RM. DC-SIGN: a guide to some mysteries of dendritic cells. *Cell* 100: 491-494, 2000.
  325. STEINMAN RM, ADAMS JC, AND COHN ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. *J Exp Med* 141: 804-820, 1975.
  326. STEINMAN RM AND COHN ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 137: 1142-1162, 1973.
  327. STEINMAN RM AND COHN ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp Med* 139: 380-397, 1974.
  328. STEINMAN RM AND GERMAIN RN. Antigen presentation and related immunological aspects of HIV-1 vaccines. *AIDS* 12: S97-S112, 1998.
  329. STEINMAN RM, KAPLAN G, WITMER MD, AND COHN ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J Exp Med* 149: 1-16, 1979.
  330. STEINMAN RM AND SWANSON J. The endocytic activity of dendritic cells. *J Exp Med* 182: 283-288, 1995.
  331. STOCKWIN LH, MCGONAGLE D, MARTIN IG, AND BLAIR GE. Dendritic cells: immunological sentinels with a central role in health and disease. *Immunol Cell Biol* 78: 91-102, 2000.
  332. STROBL H, BELLO-FERNANDEZ C, RIEDL E, PICKL WF, MAJDIC O, LYMAN SD, AND KNAPP W. flt3 ligand in cooperation with transforming growth factor-beta1 potentiates in vitro development of Langerhans-type dendritic cells and allows single-cell dendritic cell cluster formation under serum-free conditions. *Blood* 90: 1425-1434, 1997.
  333. STROBL H, SCHEINECKER C, RIEDL E, CSMARITS B, BELLO-FERNANDEZ C, PICKL WF, MAJDIC O, AND KNAPP W. Identification of CD68+lin- peripheral blood cells with dendritic precursor characteristics. *J Immunol* 161: 740-748, 1998.
  334. STUMBLES PA, THOMAS JA, PIMM CL, LEE PT, VENAILE TJ, PROKSCH S, AND HOLT PG. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 188: 2019-2031, 1998.
  335. SUBAUSTE CS AND WESSENDARP M. Human dendritic cells discriminate between viable and killed *Toxoplasma gondii* tachyzoites: dendritic cell activation after infection with viable parasites results in CD28 and CD40 ligand signaling that controls IL-12-dependent and -independent T cell production of IFN-gamma. *J Immunol* 165: 1498-1505, 2000.
  336. SUDA T, MCCARTHY K, VU Q, MCCORMACK J, AND SCHNEEBERGER EE. Dendritic cell precursors are enriched in the vascular compartment of the lung. *Am J Respir Cell Mol Biol* 19: 728-737, 1998.
  337. SUGITA M, GRANT EP, VAN DONSELAAR E, HSU VW, ROGERS RA, PETERS PJ, AND BRENNER MB. Separate pathways for antigen presentation by CD1 molecules. *Immunity* 11: 743-752, 1999.
  338. SUNG S, ROSE CE, AND FU SM. Intratracheal priming with ovalbumin and ovalbumin 323-339 peptide-pulsed dendritic cells induces airway hyperresponsiveness, lung eosinophilia, goblet cell hyperplasia, and inflammation. *J Immunol* 166: 1261-1271, 2001.
  339. SUSS G AND SHORTMAN K. A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. *J Exp Med* 183: 1789-1796, 1996.
  340. SUTER T, MALPIERO U, OTTEN L, LUDEWIG B, MUELETHALER-MOTTET A, MACH B, REITH W, AND FONTANA A. Dendritic cells and differential usage of the MHC class II transactivator promoters in the central nervous system in experimental autoimmune encephalitis. *Eur J Immunol* 30: 794-802, 2000.
  341. SWALLOW MM, WALLIN JJ, AND SHA WC. B7h, a novel costimulatory homolog of B7.1 and B7.2, is induced by TNF $\alpha$ . *Immunity* 11: 423-432, 1999.
  342. TADA Y, ASAHINA A, NAKAMURA K, TOMURA M, FUJIWARA H, AND TAMAKI K. Granulocyte/macrophage colony-stimulating factor inhibits IL-12 production of mouse Langerhans cells. *J Immunol* 164: 5113-5119, 2000.
  343. TAFURI A, SHAHINIAN A, BALDT F, YOSHINAGA SK, JORDANA M, WAKEHAM A, BOUCHER LM, BOUCHARD D, CHAN VSF, DUNCAN G, ODERMATT B, HO A, ITIE A, HORAN T, WHORISKEY JS, PAWSON T, PENNINGER JM, OHASHI PS, AND MAK TW. ICOS is essential for effective T-helper-cell responses. *Nature* 409: 105-109, 2001.
  344. TAKASHIMA A AND MORITA A. Dendritic cells in genetic immunization. *J Leukoc Biol* 66: 350-356, 1999.
  345. TAKAYAMA T, NISHIOKA Y, LU L, LOTZE MT, TAHARA H, AND THOMSON AW. Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness. *Transplantation* 66: 1567-1574, 1998.
  346. TANG HL AND CYSTER JG. Chemokine up-regulation and activated T cell attraction by maturing dendritic cells. *Science* 284: 819-822, 1999.
  347. TERMEER CC, HENNIES J, VOITH U, AHRENS T, WEISS JM, PREHM P, AND SIMON JC. Oligosaccharides of hyaluronan are potent activators of dendritic cells. *J Immunol* 165: 1863-1870, 2000.
  348. TEW JG, WU J, QIN D, HELM S, BURTON GF, AND SZAKAL AK. Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. *Immunol Rev* 156: 39-52, 1997.
  349. THOMA-USZYNSKI S, KIERTSCHER SM, OCHOA MT, BOUIS DA, NORGARD MV, MIYAKE K, GODOWSKI PJ, ROTH MD, AND MODLIN RL. Activation of toll-like receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10. *J Immunol* 165: 3804-3810, 2000.
  350. THOMSON AW AND LU L. Are dendritic cells the key to liver transplant tolerance? *Immunol Today* 20: 27-32, 1999.
  351. THOMSON AW AND LU L. Dendritic cells as regulators of immune reactivity: implications for transplantation. *Transplantation* 68: 1-8, 1999.
  352. THOMSON AW, LU L, SUBBOTIN VM, LI Y, QIAN S, RAO AS, FUNG JJ, AND STARZL TE. In vitro propagation and homing of liver-derived dendritic cell progenitors to lymphoid tissues of allogeneic recipients. Implications for the establishment and maintenance of donor cell chimerism following liver transplantation. *Transplantation* 59: 544-551, 1995.
  353. THURNER B, HAENDLE I, RODER C, DIECKMANN D, KEIKAVOUSSI P, JONULEIT H, BENDER A, MACZEK C, SCHREINER D, VON DEN DRIESCH P, BROCKER EB, STEINMAN RM, ENK A, KAMPGEN E, AND SCHULER G. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med* 190: 1669-1678, 1999.
  354. TIMMERMAN JM AND LEVY R. Dendritic cell vaccines for cancer immunotherapy. *Annu Rev Med* 50: 507-529, 1999.
  355. TINEKE CTM, KRAAN VDP, BOELE LCM, SMEENK RJT, WUDENES J, AND AARDEN LA. Prostaglandin E<sub>2</sub> is a potent inhibitor of human interleukin 12 production. *J Exp Med* 181: 775, 1995.
  356. TJOA BA AND MURPHY GP. Development of dendritic-cell based prostate cancer vaccine. *Immunol Lett* 74: 87-93, 2000.
  357. TRAYER D, AKASHI K, MANZ M, MERAD M, MIYAMOTO T, ENGLEMAN EG, AND WEISSMAN IL. Development of CD8 $\alpha$ -positive dendritic cells from a common myeloid progenitor. *Science* 290: 2152-2154, 2000.
  358. TREON SP, RAJE N, AND ANDERSON KC. Immunotherapeutic strategies for the treatment of plasma cell malignancies. *Semin Oncol* 27: 598-613, 2000.
  359. TURLEY SJ, INABA K, GARRETT WS, EBERSOLD M, UNTERNAEHRER J, STEINMAN RM, AND MELLMAN I. Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 288: 522-527, 2000.
  360. VALENTIN A, LU W, ROSATI M, SCHNEIDER R, ALBERT J, KARLSSON A, AND PAVLAKIS GN. Dual effect of interleukin 4 on HIV-1 expression: implications for viral phenotypic switch and disease progression. *Proc Natl Acad Sci USA* 95: 8886-8891, 1998.

361. VALUJSKIKH A, MATESIC D, GILLIAM A, ANTHONY D, HAQI TM, AND HEEGER PS. T cells reactive to a single immunodominant self-restricted allopeptide induce skin graft rejection in mice. *J Clin Invest* 101: 1398–1407, 1998.
362. VAN DER POEW KRAAN TC, SNLIDERS A, BOELIE LC, DE GROOT ER, ALEWJNSE AE, LEURS R, AND AARDEN LA. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J Clin Invest* 102: 1866–1873, 1998.
363. VAN ESSEN D, DULLFORCE P, BROCKER T, AND GRAY D. Cellular interactions involved in Th cell memory. *J Immunol* 165: 3640–3646, 2000.
364. VECCHI A, MASSIMILIANO L, RAMPONI S, LUINI W, BERNASCONI S, BONECHI R, ALLAVENA P, PARMENTIER M, MANTOVANI A, AND SOZZANI S. Differential responsiveness to constitutive vs. inducible chemokines of immature and mature mouse dendritic cells. *J Leukoc Biol* 66: 489–494, 1999.
365. VENDETTI S, CHAI JG, DYSON J, SIMPSON E, LOMBARDI G, AND LECHLER R. Anergic T cells inhibit the antigen-presenting function of dendritic cells. *J Immunol* 165: 1175–1181, 2000.
366. VIEIRA PL, DE JONG EC, WIERENGA EA, KAPSENBERG ML, AND KALINSKI P. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J Immunol* 164: 4507–4512, 2000.
367. VISINTIN A, MAZZONI A, SPITZER JH, WYLLIE DH, DOWER SK, AND SEGAL DM. Regulation of toll-like receptors in human monocytes and dendritic cells. *J Immunol* 166: 249–255, 2001.
368. VREMEC D, POOLEY J, HOCHREIN H, WU L, AND SHORTMAN K. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *J Immunol* 164: 2978–2986, 2000.
369. WALUNAS TL, LENSCHOW DJ, BAKKER CY, LINSLEY PS, FREEMAN GJ, GREEN JM, THOMPSON CB, AND BLUESTONE JA. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1: 405–413, 1994.
370. WANG B, CHUN T, RULIFSON IC, EXLEY M, BALK SP, AND WANG CR. Human CD1d functions as a transplantation antigen and a restriction element in mice. *J Immunol* 166: 3829–3836, 2001.
371. WANG J, RODRIGUEZ G, ORAVECZ T, AND NORCROSS MA. Cytokine regulation of human immunodeficiency virus type 1 entry and replication in human monocytes/macrophages through modulation of CCR5 expression. *J Virol* 72: 7642–7647, 1998.
372. WEINBERG AD, VELLA AT, AND CROFT M. OX-40: life beyond the effector T cell stage. *Semin Immunol* 10: 471–480, 1998.
373. WEISSMAN D, NI H, SCALES D, DUDE A, CAPODICI J, MCGIBNEY K, ABDOL A, ISAACS SN, CANNON G, AND KARIKO K. HIV gag mRNA transfection of dendritic cells (DC) delivers encoded antigen to MHC class I and II molecules, causes DC maturation, and induces a potent human in vitro primary immune response. *J Immunol* 165: 4710–4717, 2000.
374. WELLS AD, LI XC, LI Y, WALSH MC, ZHENG XX, WU Z, NUNEZ G, TANG A, SAYEGH M, HANCOCK WW, STROM TB, AND TURKA LA. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. *Nat Med* 5: 1303–1307, 1999.
375. WERTS C, TAPPING RI, MATHISON JC, CHUANG TH, KRAVCHENKO V, SAINT GIRONS I, HAAKE DA, GODOWSKI PJ, HAYASHI F, OZINSKY A, UNDERHILL DM, KIRSCHNING CJ, WAGNER H, ADEREM A, TOBIAS PS, AND ULEVITCH RJ. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2: 346–352, 2001.
376. WHELAN M, HARNETT MM, HOUSTON KM, PATEL V, HARNETT W, AND RIGLEY KP. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J Immunol* 164: 6453–6460, 2000.
377. WHITTAKER DS, BAHJAT KS, MOLDAWER LL, AND CLARE-SALZLER MJ. Autoregulation of human monocyte-derived dendritic cell maturation and IL-12 production by cyclooxygenase-2-mediated prostanoïd production. *J Immunol* 165: 4298–4304, 2000.
378. WINKEL KD, KRONIN V, KRUMMEL MF, AND SHORTMAN K. The nature of the signals regulating CD8 T cell proliferative responses to CD8 $\alpha$ <sup>+</sup> or CD8 $\alpha$ <sup>−</sup> dendritic cells. *Eur J Immunol* 27: 3350–3359, 1997.
379. WOO J, LU L, RAO AS, LI Y, SUBBOTIN V, STARZL TE, AND THOMSON AW. Isolation, phenotype, and allostimulatory activity of mouse liver dendritic cells. *Transplantation* 58: 484–491, 1994.
380. WU L, D'AMICO A, WINKEL KD, SUTER M, LO D, AND SHORTMAN K. RelB is essential for the development of myeloid-related CD8 $\alpha$ <sup>−</sup> dendritic cells but not of lymphoid-related CD8 $\alpha$ <sup>+</sup> dendritic cells. *Immunity* 9: 839–847, 1998.
381. WU L, LI CL, AND SHORTMAN K. Thymic dendritic cell precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. *J Exp Med* 184: 903–911, 1996.
382. WU L, NICHOGIANNPOULOU A, SHORTMAN K, AND GEORGIOPOULOS K. Cell-autonomous defects in dendritic cell populations of Ikaros mutant mice point to a developmental relationship with the lymphoid lineage. *Immunity* 7: 483–492, 1997.
383. WYKES M, POMBO A, JENKINS C, AND MACPHERSON GG. Dendritic cells interact directly with naïve B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J Immunol* 161: 1313–1319, 1998.
384. YAMAGUCHI Y, TSUMURA H, MIWA M, AND INABA K. Contrasting effects of TGF- $\beta$ 1 and TNF- $\alpha$  on the development of dendritic cells from progenitors in mouse bone marrow. *Stem Cells* 15: 144–153, 1997.
385. YANG D, CHEN Q, STOLL S, CHEN X, HOWARD OM, AND OPPENHEIM JJ. Differential regulation of responsiveness to fMLP and C5a upon dendritic cell maturation: correlation with receptor expression. *J Immunol* 165: 2694–2702, 2000.
386. YANG JS, XU LY, HUANG YM, VAN DER MEIDE PH, LINK H, AND XIAO BG. Adherent dendritic cells expressing high levels of interleukin-10 and low levels of interleukin-12 induce antigen-specific tolerance to experimental autoimmune encephalomyelitis. *Immunology* 101: 397–403, 2000.
387. YANG OO, RACKE FK, NGUYEN PT, GAUSLING R, SEVERINO ME, HORTON HF, BYRNE MC, STROMINGER JL, AND WILSON SB. CD1d on myeloid dendritic cells stimulates cytokine secretion from and cytolytic activity of Valpha24JalphaQ T cells: a feedback mechanism for immune regulation. *J Immunol* 165: 3756–3762, 2000.
388. YOSHIDA R, NAGIRA M, KITaura M, IMAGAWA N, IMAI T, AND YOSHIE O. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. *J Biol Chem* 273: 7118–7122, 1998.
389. YOSHINAGA SK, WHORISKEY JS, KHARE SD, SARMIENTO U, GUO J, HORAN T, SHIH G, ZHANG M, COCCIA MA, KOHNO T, TAFURI-BLADT A, BRANKOW D, CAMPBELL P, CHANG D, CHIU L, DAI T, DUNCAN G, ELLIOTT GS, HUI A, MCCABE SM, SCULLY S, SHAHINIAN A, SHAKLEE CL, VAN G, MAK TW, AND SENALDI G. T-cell co-stimulation through B7RP-1 and ICOS. *Nature* 402: 827–832, 1999.
390. YU C, SEIDEL K, NASH RA, DEEG HJ, SANDMAIER BM, BARSOUKOV A, SANTOS E, AND STORR R. Synergism between mycophenolate mofetil and cyclosporine in preventing graft-versus-host disease among lethally irradiated dogs given DLA- nonidentical unrelated marrow grafts. *Blood* 91: 2581–2587, 1998.
391. ZENG Z, CASTANO AR, SEGELKE BW, STURA EA, PETERSON PA, AND WILSON IA. Crystal structure of mouse CD1: an MHC-like fold with a large hydrophobic binding groove. *Science* 277: 339–345, 1997.
392. ZHANG Y, ZHANG YY, OGATA M, CHEN P, HARADA A, HASHIMOTO S, AND MATSUSHIMA K. Transforming growth factor- $\beta$ 1 polarizes murine hematopoietic progenitor cells to generate Langerhans cell-like dendritic cells through a monocyte/macrophage differentiation pathway. *Blood* 93: 1208–1220, 1999.
393. ZHOU LJ AND TEDDER TF. CD14<sup>+</sup> blood monocytes can differentiate into functionally mature CD83<sup>+</sup> dendritic cells. *Proc Natl Acad Sci USA* 93: 2588–2592, 1996.
394. ZINKERNAGEL RM AND ALTHAGE A. On the role of thymic epithelium vs. bone marrow-derived cells in repertoire selection of T cells. *Proc Natl Acad Sci USA* 96: 8092–8097, 1999.
395. ZITVOGEL L, REGNAULT A, LOZIER A, WOLFERS J, FLAMENT C, TENZA D, RICCIARDI-CASTAGNOLI P, RAPOSO G, AND AMIGORENA S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 4: 594–600, 1998.
396. ZLOTNIK A AND YOSHIE O. Chemokines: a new classification system and their role in immunity. *Immunity* 12: 121–127, 2000.
397. ZOETEWEIJ JP, GOLDING H, MOSTOWSKI H, AND BLAUVELT A. Cytokines regulate expression and function of the HIV coreceptor CXCR4 on human mature dendritic cells. *J Immunol* 161: 3219–3223, 1998.